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(54) Title: THE TRANSMEMBRANE PROTEIN AMIGO AND USES THEREOF

(57) Abstract: Tranmembrane proteins AMIGO, AMIGO2 AND AMIGO3 (Amphoterin induced gene and orphan receptor), wherein the extracellular part contain six leucine-rich repeats (LRRs) and one immunoglobulin domain. Use of said proteins for modulating growth, migration, axonal growth, myelinatino, fasciculation or proliferation of neuronal cells and for treating cancer, tumour growth or metastasis. Methods of screening for agents which modulate the interaction between two AMIGO compounds or between AMIGO and epidermal growth factor receptor (EGFR).

Novel protein and uses thereof

FIELD OF THE INVENTION

The present invention generally relates to the field of genetic engineering and more particularly to transmembrane proteins implicated in axon tract development.

BACKGROUND OF THE INVENTION

Development of the nervous system with billions of connections is one of the most complex and fascinating phenomena in nature. One key feature in this event is the guidance of the neuronal growth cones to their appropriate targets. A wide variety of soluble matrix and cell surface molecules have been found to be involved in axonal growth and in association of axons to form mature fiber tracts (for reviews, see Tessier-Lavigne and Goodman, 1996; Drescher et al., 1997).

Within the peripheral nervous system (PNS), injured nerve fibers can regrow over long distances, with eventual excellent recovery of function. Within the past 15 years, neuroscientists have come to realize that this is not a consequence of intrinsic differences between the nerve cells of the peripheral and central nervous system; remarkably, neurons of the CNS will extend their axons over great distances if given the opportunity to grow through a grafted segment of PNS (e.g., sciatic nerve). Therefore, neurons of the CNS retain a capacity to grow if given the right signals from the extracellular environment. Factors which contribute to the differing growth potentials of the CNS and PNS include partially characterized, growth-inhibiting molecules on the surface of the oligodendrocytes that surround nerve fibers in the CNS, but which are less abundant in the comparable cell population of the PNS (Schwann cells); molecules of the basal lamina and other surfaces that foster growth in the PNS but which are absent in the CNS (e.g., laminin); and trophic factors, soluble polypeptides which activate programs of gene expression that underlie cell survival and differentiation. Although such trophic factors are regarded as essential for maintaining the viability and differentiation of nerve cells, the particular ones that are responsible for inducing axonal regeneration in the CNS remain uncertain. As a result, to date, effective treatments for CNS injuries have not been developed.

Immunoglobulin superfamily proteins form the most diverse and studied class of molecules, which have been shown to participate in contact-dependent regulation of neurite outgrowth, axon guidance and synaptic plasticity (for reviews see, Schachner, 1997; Walsh and Doherty, 1997; Stoeckli and Landmesser, 1998; Van Vactor, 1998). Extracellular proteins containing leucine-rich repeats (LRRs) have also been shown to participate in axon guidance. For instance, Slit proteins containing LRR domains act as midline repellents for commissural axons through the Robo (Roundabout) receptor (Battye et al., 1999; Brose et al., 1999) and recently Battye et al. (2001) showed that the interaction of Slits with their Robo receptors was due to LRRs found in Slits. Furthermore, Pusch et al. (2000) showed that the disease called X-linked congenital stationary night blindness (XLCSNB) maps to a gene, which codes only the LRR containing protein Nyctalopin in retina. Recently the receptor for axonal regeneration inhibitor Nogo (Chen et al., 2000) was found to be a GPI-linked cell surface protein where the only recognizable motifs are LRR domains (Fournier et al., 2001).

Amphoterin (also known as HMGB1) is a heparin-binding protein that was isolated from perinatal rat brain as a neurite outgrowth-promoting factor (Rauvala and Pihlaskari, 1987) enriched in the growth cones of neuronal cells. Amphoterin has been proposed to be an autocrine factor in invasive cell or growth cone migration due to binding to the cell surface receptors (RAGE and sulphated glycan epitopes) and to activation of proteolysis of ECM through binding of plasminogen and it's activators to amphoterin (for reviews see Rauvala et al., 2000; Muller et al., 2001).

To examine the role of amphoterin in cell motility, especially in neurite outgrowth, we searched for genes that are induced on amphoterin matrix by using mRNA differential display. In this invention, we describe the cloning and functional characterization of a novel protein named as AMIGO (AMphoterin Induced Gene and Orphan receptor). Cloning of AMIGO gave us sequence data to clone two other related proteins (AMIGO2 and AMIGO3); together these three proteins form a novel family of transmembrane proteins. The predicted amino acid sequences of the AMIGOs suggest that they are type I transmembrane proteins containing a signal sequence for secretion and a transmembrane domain. Interestingly, the extracellular part of the AMIGOs contains six leucine-rich repeats (LRRs) flanked by cysteine-rich LRR N- and C-terminal domains and one immunoglobulin domain close to the transmembrane region. This twin motif structure

defines the AMIGOs as members of both the immunoglobulin and the leucine-rich repeat superfamilies.

Amphoterin

Amphoterin is a protein, which was isolated from perinatal rat brain according to its ability to promote neurite outgrowth (Rauvala and Pihlaskari, 1997). Amphoterin is a dipolarised molecule, which contains both positively and negatively charged regions. This dipolar nature of amphoterin renders it very adhesive molecule, which binds for example to heparin and other sulphated glycans.

Amphoterin is also found to localize in nucleus and to bind DNA and in this role it is called as HMG1 (Bianchi et al., 1989). In subsequent studies amphoterin has been shown to localize diffusibly inside the cell but when the cell starts to grow projections amphoterin is localized into the tips of the projections (Merenmies et al., 1991; Parkkinen et al., 1993). Although amphoterin lacks the signal sequence for secretion, it has been shown to be present also in the extracellular matrix (ECM). In vitro amphoterin has been shown to localize to the surface of the neurons (Rauvala and Pihlaskari, 1997; Rauvala et al., 1988) and amphoterin has been shown to be a ligand for the cell surface receptor RAGE (Hori et al., 1995). During the endotoxin shock large quantities of amphoterin has been shown to accumulate into the human plasma (Wang et al., 1999). During the period when the red blood cells are maturing amphoterin is secreted into the ECM, where it is believed to work as a differentiation factor (Passalacqua et al., 1997). It is also suggested that the amphoterin secreted from glial cells works as a factor between the interaction of glia and neurons (Passalacqua et al., 1998; Daston and Ratner, 1994).

Amphoterin is highly expressed in neurons and glial cells in developing nervous system and generally in non-mature cells. Amphoterin is also highly expressed in monocytes and macrophages and often in transformed cells. Amphoterin is thought to be involved in invasive migration of cells. Amphoterin binds plasminogen and plasminogen activators and this binding has been shown to activate the formation of the plasmin and also degradation of amphoterin (Parkkinen and Rauvala, 1991; Parkkinen et al., 1993). At the cell surface level amphoterin binds to the transmembrane protein RAGE and some proteoglycans (like Syndecan-1) and sulphoglycolipids. The multiligand protein RAGE (Receptor of advanced glycation end products) is a member of immunoglobulin

superfamily. Amphoterin stimulates the neurite outgrowth via RAGE dependent signalling and the both proteins also localize in same areas of the developing nervous system (Hori et al., 1995). It has been suggested that amphoterin works as an autocrine and/or paracrine factor in invasive migration; amphoterin binds to its receptors and activates both the proteolysis of the ECM and the reorganization of the cytoskeleton (Rauvala et al., 2000; Rauvala et al., 1988). It has been shown that by inhibiting the interaction between amphoterin and RAGE the growth and the invasiveness of the tumour could be reduced.

Immunoglobulin domains

IgG domain is one of the most common extracellular protein motifs. It was first discovered from antibody molecules. In addition of antibody molecules, many cell adhesion molecules, cell surface receptors and some intracellular muscular proteins contain IgG domains. IgG domain is about 70-110 amino acids long usually containing two cysteines separated by 55-77 amino acids, it forms 7-10 beta sheets, and is a tightly packed globular structure with hydrophobic residues inside and hydrophilic outside. The structure is often stabilized by disulfide bridge between conserved cysteines (Walsh and Doherty 1997; Williams and Barclay 1988).

In sequence level IgG domains differ greatly. The homology between different IgG domains within the same protein may share only 10-30 % amino acid similarity. Although all IgG domains share the same core structure, two beta sheets stacked together, the other features can vary considerably. In spite of variability within IgG domains they can be classified in categories. Originally they were classified as C1, C2 and V, and later Group I was added (Williams and Barclay 1988). The stability of IgG domain may explain why it commonly resides in extracellular space, it is resistant to proteolytic and oxidative environment. Extracellular IgG domain containing molecules may function in cell adhesion and in recognition and binding of molecules. IgG domain seems to interact with any parts of its domain surface. (Williams and Barclay 1988)

IgG domain containing proteins form so called immunoglobuling superfamily of proteins, which is the most common family of cell surface proteins. Sequence analysis has shown that 765 human proteins belong to this family, in flyes there are 140 and in worms 64 proteins (Venter et al 2001). The members of IgG family encode proteins that are involved in cell recognition and adhesion such as antibody molecules, T-cell receptors, growth

factor receptors, many adhesion molecules and neurite outgrowth promoting receptors. IgG domain adhesion molecules often consist of several consequent IgG domains and type III fibronectin like domains (Crossin and Krushel 2000).

Neuronal members of immunoglobuling superfamily act as receptor and adhesion molecules and their role have been especially indicated in many important functions related to axonal growth and guidance. Adhesion molecules have important roles during the neuronal development where many interactions need to be coordinated in precise manner, for example NCAM and L1 which function during axonal growth and guidance (Walsh and Doherty 1997). Other members include receptor for FGF (FGFR, Trk family of neurotrophic factor receptors, Eph receptors, Robo (Roundabout) that mediates the functions of Slit and DCC (Deleted in colorectal carcinoma) that interacts with netrins (Tessier-Lavigne and Goodman 1996; Brose and Tessier-Lavigne 2000).

Axonal IgG cell adhesion molecules may interact in homophilic or heterophilic way with other IgG family members. The binding partner may localize at the same cell membrane, in adjacent cell membrane or in extracellular space. Many IgG proteins form a very complex network of cellular interactions where they can even have partially overlapping functions. They may also compete for the same ligands by modulating their binding affinity to other ligands (Brummendorf and Lemmon 2001).

Immunoglobulin superfamily members involved in myelinization are MAG (myelin-associated glycoprotein) and P0 although their precise actions are not known. MAG's functions have been shown to be associated with inhibition of regeneration of CNS neurons or it can either activate or inhibit the neurite growth of certain neurons. Approximately half of the all protein in myelin consists of P0 protein which is a homophilic cell adhesion molecule thought to be involved in interconnection of cell membranes of myelin sheath (Brummendorf and Rathjen 1994).

LRR domains

Leucine rich repeats (LRR) are 20-29 amino acid long sequence motifs characterized by repetition of hydrophobic residues, especially leucine and that are separated by conserved distance. The sequence repeat is found in several times in protein and this region of repeats is called LRR domain. LRR contains conserved 11 amino acid long consensus sequence,

LxxLxLxxzxL where x stands for any amino acid, z for N or cysteine and L for leucine, valine, isoleucine or phenylalanine. LRR proteins contain usually many LRR domains and can contain up to 30 repeats (chaoptin). LRR domains are not always identical to consensus sequence and may therefore contain gaps, have different lengths or amino acid compositions (Kobe and Deisenhofer 1994).

To prevent the sole hydrophobic core of LRR domain from interacting directly with solvents it is flanked by several cysteine residues at its N and/or C terminal sides (LRRNT, LRRCT domains). Sequence analysis has revealed that there are four different C terminal cysteine rich domains and one N terminal one (Kobe and Kajava 2001; Kajava 1998). These cysteine domains are only found from extracellular proteins and cysteines form intermolecular disulfide bridges (Kresse et al 1993; Hashimoto et al 1991).

LRR domain proteins are located in various places in cells and they have different functions. Eukaryotic LRR proteins can be found in nucleus, cytoplasm, cell membrane as well as in extracellular space and they can act as hormone receptors, subunits of enzymes, cell adhesion molecules and in cell recognition (Kobe and Kajava 2001). Moreover, they mediate various cellular functions such as signal transduction, intracellular transport, and DNA repair, recombination and transcription (Buchanan and Gay 1996).

LRR proteins can be divided into at least 7 different subclasses according to the length of LRR and the composition of consensus sequence. Subclasses are RI-like, SDS22-like, cysteine containing, bacterial, typical, plant specific and TpLRR (Kobe and Kajava 2001). Former three are intracellular whereas latter four are found in cellular membranes or in the extracellular space.

LRR domains are thought to have a role in protein-protein interactions. For example, chaoptin is a cell surface protein which consists of LRR domains, is attached to cell membrane via lipid anchor, and has been shown to mediate homophilic cell adhesion (Reinke et al 1988; Krantz and Zipursky 1990).

Extracellular matrix contains several homologous small proteglycans whose sequences are composed 70-80% of LRR domains. These small proteoglycans are composed of N-terminal glycosaminoglycans and variable amounts of LRRs that are flanked by LRRNT

and LRRCT domains. Proteoglycans such as biglycan binds to laminin and fibronectin whereas decorin and fibromodulin bind to type I and II collagens (Svensson et al 1995). Axonal growth modulating molecule Slit contains LRR domain, EGF repeats, laminin like G domain and LRRNT and LRRCT domains. Only LRR domain of Slit is needed for its binding to Robo in vitro as well as mediating repulsive signalling in vivo (Battye et al 2001).

Although several LRR proteins are expressed in the nervous system only few of those functions or binding partners are known. The best characterized neuronal LRR proteins are Drosophila's connectin, capricious and chaoptin. Connectin is a GPI (glycosyl phosphatidyl inositol) linked cell adhesion protein that has a role during the development of neuromuscular junction. It contains 10 LRR domains flanked by LRRCT domain. During the formation of neuromuscular junction connectin is expressed in surfaces of certain muscle cells and concomitantly in their innervating motor neurons where the expression is especially seen in growth cones. During synapse formation connectin localizes in junctional areas but during synapse maturation connectin expression is downregulated. In vitro experiments have indicated increased homophilic cell adhesion between connectin transfected S2 cells (Nose et al 1992; Meadows et al 1994). Moreover, in vivo studies have supported its role as attractive neuronal growth modulating protein. When connectin is misexpressed in all muscular cells aberrant neuromuscular junction formation occurs (Yu et al 2000).

Capricious is a cell membrane protein sharing similarities with the functions of connectin in neuromuscular junction formation. It contains 12 LRR domains flanked by LRRCT and LRRNT domains. It probably mediates cell-to-cell signalling processes during the formation of the neuromuscular junction since in vitro studies have not supported its homophilic adhesion (Shishido et al 1998).

Chaoptin is a photoreceptor cell specific adhesion molecule which contains 30-40 LRR domains and is linked to cell membrane via GPI anchor. It mediates homophilic cell adhesion and is needed for the proper formation of a photoreceptor cell (Krantz and Zipursky 1990).

Slit proteins are conserved, secreted into extracellular space and provide guidance during axonal growth and branching. Slit proteins consist of several LRR domains, EGF like repeats, laminin like G-domain and LRRNT domain (Brose and Tessier-Lavigne 2000). Slit was discovered from fruit fly where it repels axonal growth (Rothberg et al 1990; Kidd et al 1999). Slit is produced by glial cells of midline and it is needed for the formation of axonal tracts crossing the midline as well as positioning of horizontal lateral tracts. Biological functions of Slit are mediated by Robo which is a cell membrane receptor. LRR domains of Slit bind to Robo in vitro and LRR domains are needed for Slit's repulsive signalling (Battye et al 2001). Three mammalian Slits and Robos have been cloned. In addition, Slit binds to laminin-1, netrin-1, and glypican-1 (Brose et al 1999; Liang et al 1999).

Nogo receptor (NogoR) is CNS receptor protein found in myelin and responsible for the inhibition of axonal regeneration. NogoR consists of 8 LRR domains flanked by LRRCT domain and it is attached to cell membrane via GPI anchor. It binds to Nogo-66 while inhibiting axonal growth (Grandpre and Strittmatter 2001; Fournier et al 2001).

OMgp is oligodendrocyte-myelin glycoprotein found in CNS myelin and cell membranes of oligodendrocytes. It is 110 kDa GPI-anchored cell membrane protein containing at least 6 LRR domains and LRRNT domain (Mikol et al 1988 and 1990). Like Nogo, OMgp inhibits axonal regeneration in the mammalian CNS. Until recently OMgp has been shown to bind NogoR while inhibiting axonal regeneration (Wang et al 2002).

LRR- and Ig-domains containing proteins

Some transmembrane proteins of nervous system contain both LRR- and Ig-domains, which are discussed below.

Kekkon and ISLR

Drosophilae (fruit fly) has gene family called *kekkon,* which codes transmembrane proteins with both LRR- and Ig-domains. The extracellular part of the kekkon1 (kek1) and kekkon2 (kek2) contains six LRRs flanked with LRRNT and LRRCT domains. They also contain one type C2 Ig-domain close to transmembrane region and large intracellular tail. Both genes are expressed in developing central nervous system (CNS) and the kekkon1 is also present in developing ovary (Musacchio and Perrimon, 1996).The kek1 has been shown to

inhibit the function of the epidermal growth factor receptor (EGFR) in oogenesis (Ghiglione, 1999). Interestingly, only the extracellular part and transmembrane domains of the kek1 protein are needed for EGFR inhibition.

The transmembrane protein ISLR has same kind of domain structure as the kekkon proteins. The extracellular part of the ISLR contains six LRRs lined with LRRNT and LRRCT domains. It also contains one type C2 Ig-domain close to transmembrane region but it does not contain intracellular part. The ISLR has been cloned from humans and mice. The ISLR is expressed in various tissues like retina, heart, thymus and spinal cord (Nagasawa et al., 1999; Nagasawa et al., 1997).

Trk-receptors

Neurotrophin receptors TrkA, TrkB and TrkC are receptor tyrosine kinases, in which the extracellular part contains three LRR-areas and each area is flanked with LRRNT and LRRCT domains. The extracellular part contains also two Ig- domains. The intracellular parts of Trk-receptors contain tyrosine kinase domain. The ligands of Trk-receptors are neurotrophins, which are important factors in development and in maintenance of central and peripheral nervous system. The binding of the neurotrophins into the Trk-receptor dimerazes the receptor and the tyrosine kinase domain is autophosphorylated and this phosphorylation activates several signalling cascades (Kaplan and Miller, 1997). Originally, some studies indicated that the LRR-areas of the Trk-receptors are the ligand binding domains (Windisch et al., 1995; Windish et al., 1995). Recently it has been shown that the Ig-domain of the TrkA receptor closest to the cell surface is the one, which binds the Nerve growth factor (NGF)(Holden et al., 1997; Perez et al., 1995; Robertson et al., 2001; Urfer et al., 1995; Wiesmann et al., 1999).

NLRRs, Pal and LIG-1

Neuronal Leucine-rich repeat proteins (NLRRs) are transmembrane proteins expressed in nervous tissues. The extracellular part of the NLRRs contains 12 LRRs flanked with LRRNT and LRRCT domains, one Ig-domain and type III fibronectin like domain. Similar NLRR proteins have been found from mouse, rat, zebra fish, frog and human (Hayata et al., 1998; Taniguchi et al., 1996: Taguchi et al., 1996; Bormann et al., 1999; Fukamachi et al., 1998). In zebra fish one member of NLRR family is expressed specifically during the axonal regeneration after injury (Hayata et al., 1998). Unlike in adult mammalian CNS the

neurons of the fish could raise new neurons into the injured area. In mouse NLRR-3 gene has been shown to be induced after cortical injury (Ishii et al., 1996).

Pal is a transmembrane protein, which is expressed specifically in retina. The extracellular part of the pal contains five LRRs flanked with LRRNT and LRRCT domains, one type C2 Ig-domain and type III fibronectin like domain. In adult retina pal is expressed by photoreceptor cells, where protein is believed to localize in disks. The function of the pal is not yet known, but it has been shown to form homodimers (Gomi et al., 2000).

LIG-1 is also a transmembrane protein, which contains both LRRs and immuoglobulin domains. The extracellular part of the LIG-1 contains 15 LRRs and three type C2 Ig-domains. The intracellular part of the LIG-1 is 270 amino acids long and it does not contain any known domains. LIG-1 is expressed highly in brain both in mice and humans. In mouse the LIG-1 expression is localized in particular subpopulations of neuronal support cells; in cerebellum LIG-1 is localized in Bergman glia cells (Nilsson et al., 2001; Suzuki et al., 1996).

In this invention we have characterized AMIGO, AMIGO2 and AMIGO3, the members of the protein family that is highly expressed in the nervous system. We disclose that AMIGOs mediate cell-to-cell interactions via a homophilic and heterophilic mechanism during the development of the fiber tracts of the nervous system.

Epidermal Growth Factor Receptor

Epidermal growth factor receptor (EGFR) is a 170 kDa transmembrane glycoprotein which possesses the intrinsic tyrosine kinase activity (Cohen et al., 1982). EGFR exerts a great variety of biological functions including cell survival, mitogenic response, differentiation and cell motility (Khazaie et al., 1993). Many ligands for EGFR have been identified including epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), amphiregulin (AR), epiregulin (EP), Batacellulin (BTC), Heparin-binding EGF-like growth factor (HB-EGF) and Schwannoma-derived growth factor (SDGF). The EGF-family of peptides is significantly involved in the regulation of mammary-gland development, morphogenesis and lactation, and also implicated in the pathogenesis of human breast cancer (Normanno and Ciardiello, 1997).

Epidermal Growth Factor Receptor (EGFR) (SEQ ID NOS:21-24) is a specific receptor for epidermal growth factor (EGF) (SEQ ID NOS:25-28) and transforming growth factor-α (TGF-α) (SEQ ID NOS:29-32). When these mitogenic polypeptides bind to EGFR, tyrosine kinase activity of the receptor is induced, and this in turn triggers a series of events which regulate cell growth. A number of malignant and non-malignant disease conditions are now believed to be associated with EGFR, particularly aberrant expression of EGFR. Aberrant expression includes both increased expression of normal EGFR and expression of mutant EGFR. Overexpression of EGFR is found in many human tumors including most glioblastomas and breast, lung, ovarian, colorectal, bladder, pancreatic, squamous cell and renal carcinomas. Elevated EGFR levels correlate with poor prognosis in human tumors. The sequence of the mRNA encoding human EGFR is known (Ullrich et al., Nature, 1984, 309, 418; GenBank Accession Number NM_005228). The gene encoding EGFR is also known as c-erb-B1. Two EGFR transcripts typically appear on Northern blots, one measuring 10 kb and one measuring 5.6 kb.

One role the EGF receptor system may play in the oncogenic growth of cells is through autocrine-stimulated growth. If cells express the EGFR and secrete EGF and/or TGF-α then such cells could stimulate their own growth. Since some human breast cancer cell lines and tumors express EGFR (Osborne, et al., J. Clin. Endo. Metab., 55:86-93 (1982); Fitzpatrick, et al., Cancer Res., 44:3442-3447 (1984); Filmus, et al., Biochem. Biophys. Res. Commun., 128:898-905 (1985); Davidson, et al., Mol. Endocrinol., 1:216-223 (1987); Sainsbury, et al., Lancet, i: 1398-1402 (1987); Perez, et al., Cancer Res. Treat., 4:189-193 (1984)) and secrete TGF.alpha. A (Bates, et al., Cancer Res., 46:1707-1713 (1986); Bates, et al., Mol. Endocrinol., 2:543-555 (1988)), an autocrine growth stimulatory pathway has been proposed in breast cancer (Lippman, et al., Breast Cancer Res. Treat., 7:59-70 (1986)).

A number of inhibitors of EGFR have been shown to be effective in inhibiting the growth of human tumor cells. Monoclonal antibodies to EGFR and drugs which inhibit EGFR tyrosine kinase activity can inhibit the growth of human cancer cell xenografts in nude mice. Normanno et al., Clin. Cancer Res., 1996, 2, 601 and Grünwald et al, J Nat Cancer Inst, 2003, 95:851. The drug PD153035, which inhibits EGFR tyrosine kinase activity, can inhibit the growth of A431 cells in nude mice, and tyrphostins, which inhibit the activity of

EGFR as well as other tyrosine kinases, have been shown to inhibit the growth of squamous carcinoma in nude mice. Kunkel et al., Invest. New Drugs, 1996, 13, 295 and Yoneda et al., Cancer Res., 1991, 51, 4430. Additonal small molecule tyrosine kinase inhibitors include ZD1839, OSI-774, CI-1033, PKI-166, GW2016, EKB-569, PD168393, AG-1478, and CGP-59326A (Grünwald et al, J Nat Cancer Inst, 2003, 95:851 incorporated herein by reference in the entirety.

Furthermore, EGFR expression is frequently accompanied by the production of EGFR-ligands, TGF-α and EGF among others, by EGFR-expressing tumor cells which suggests that an autocrine loop participates in the progression of these cells (Baselga, et al. (1994) Pharmac. Therapeut. 64:127-154; Modjtahedi, et al. (1994) Int. J. Oncology. 4:277-296). Blocking the interaction between such EGFR ligands and EGFR therefore can inhibit tumor growth and survival (Baselga, et al. (1994) Pharmac. Therapeut. 64:127-154).

A variety of approaches can be used to target EGFR such as using monoclonal antibodies to compete with the binding of activating ligands to the extracellular domain of the receptor, using small molecule inhibitors ot the intracellular tyrosine kinase domain of the receptor, using immunotoxin conjugates to deliver toxins that target EGFR to tumour cells, reducing the level of EGFR through the use of antisense oligonucleotides, and inhibiting downstream effectors of the EGFR signalling network. Despite the foregoing approaches, the need exists for new compounds against EGFR which are effective at treating and/or preventing diseases related to expression of EGFR.

SUMMARY OF THE INVENTION

The present invention provides methods and compositions relating to vertebrate AMIGO, AMIGO2, AMIGO3, collectively vertebrate AMIGO polypeptides, related nucleic acids, and polypeptide domains thereof having vertebrate AMIGO-specific structure and activity, and modulators of vertebrate AMIGO function. Vertebrate AMIGO polypeptides can regulate cell, especially nerve cell, function and morphology. The polypeptides may be produced recombinantly from transformed host cells from the subject vertebrate AMIGO polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated vertebrate AMIGO hybridization probes and primers capable of

specifically hybridizing with natural vertebrate AMIGO genes, vertebrate AMIGO-specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for vertebrate AMIGO transcripts), therapy (e.g. to modulate nerve cell growth) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating vertebrate AMIGO genes and polypeptides and reagents for screening chemical libraries for lead pharmacological agents.

In one embodiment, the invention contemplates in vitro methods and kits for culturing neuronal cells under conditions where the subject polypeptides are used to promote neurite outgrowth, and can include methods for detecting the presence and amount of stimulation of neurite outgrowth in the cultured neuronal cells. AMIGO proteins and polypeptides disclosed herein are useful according to the within-disclosed methods and may be included in the kits that are also described herein.

Appropriate cells are prepared for use in a neurite outgrowth assay. For example, a preparation of hippocampal neurons is disclosed in the Examples. Before beginning the assay, the cells may be resuspended, added to substrate-coated dishes, and placed under predetermined assay conditions for a preselected period of time. After the attachment and growth period, the dishes may be rinsed to remove unbound cells, fixed, and viewed--e.g., by phase contrast microscopy.

Preferably, a plurality of cells are analyzed for each substrate. Cells are then "judged" based on predetermined criteria. For example, cells may be considered neurite-bearing if the length of the processes are greater than one cell diameter. The percent of cells that are sprouting neurites is preferably determined, as is the average neurite length. A particularly preferred neurite outgrowth assay method is disclosed in the Examples.

The proteins and polypeptides of the present invention are therefore useful in a variety of applications relating to cell and tissue cultures.

For example, in one embodiment, a method of inhibiting neurite outgrowth of neuronal cells in a cell culture system comprises the steps of (1) introducing neuronal cells into tissue culturing conditions comprising a culture medium; and (2) introducing an AMIGO polypeptide of the present invention into the culture medium in an amount effective to

inhibit neurite outgrowth in the culture.

In another embodiment, a method of promoting neurite outgrowth of neuronal cells in a cell culture system comprises the steps of (1) immobilizing on the substrate a polypeptide of the present invention having neurite outgrowth-promoting activity; and (2) contacting neuronal cells with the substrate under tissue culturing conditions.

In another embodiment, a method of promoting neurite outgrowth of neuronal cells in a cell culture system comprises the steps of (1) introducing an AMIGO nucleic acid encoding peptide having neurite outgrowth-promoting activity of the present invention; (2) immobilizing on the substrate a polypeptide of the present invention having neurite outgrowth-promoting activity; and (3) culturing said neuronal cells under tissue culturing conditions.

The invention also discloses compositions comprising polypeptides exhibiting a neurite outgrowth-promoting in substantially pure form. In various embodiments, the polypeptides are derived from segments of an AMIGO protein.

In another embodiment, a composition according to the present invention comprises a subject polypeptide in substantially pure form and attached to a solid support or substrate. The solid support may be a prosthetic device, implant, or suturing device designed to have a surface in contact with neuronal cells or the like; further, it may be designed to lessen the likelihood of immune system rejection, wherein said surface of said device is coated with a subject polypeptide or other material designed to ameliorate rejection.

The AMIGO proteins, polypeptides, and nucleid acids disclosed herein are also useful in a variety of therapeutic applications as described herein.

The present therapeutic methods are useful in treating peripheral nerve damage associated with physical or surgical trauma, infarction, toxin exposure, degenerative disease, malignant disease that affects peripheral or central neurons, or in surgical or transplantation methods in which new neuronal cells from brain, spinal cord or dorsal root ganglia are introduced and require stimulation of neurite outgrowth from the implant and innervation into the recipient tissue. Such diseases further include but are not limited to

CNS lesions, gliosis, Parkinson's disease, Alzheimer's disease, neuronal degeneration, and the like. The present methods are also useful for treating any disorder which induces a gliotic response or inflammation.

In treating nerve injury, contacting a therapeutic composition of this invention with the injured nerve soon after injury is particularly important for accelerating the rate and extent of recovery.

Thus the invention contemplates a method of promoting neurite outgrowth in a subject, or in selected tissues thereof, comprising administering to the subject or the tissue a physiologically tolerable composition containing a therapeutically effective amount of a neurite outgrowth-promoting AMIGO compound of the present invention.

In preferred methods, a human patient is the subject, and the administered polypeptide comprises extracellular domain of human AMIGO. In anothere preferred method, a human patient is the subject, and the administered nucleic acid encodes AMIGO extracellular domain of human AMIGO.

In one embodiment, a severed or damaged nerve may be repaired or regenerated by surgically entubating the nerve in an entubalation device in which an effective amount of a neurite outgrowth-promoting polypeptide of this invention can be applied to the nerve.

In a related embodiment, a polypeptide of the invention can be impregnated into an implantable delivery device such as a cellulose bridge, suture, sling prosthesis or related delivery apparatus. Such a device can optionally be covered with glia, as described by Silver, et al, Science 220:1067-1069, (1983), which reference is hereby incorporated by reference.

Therapeutic compositions of the present invention may include a physiologically tolerable carrier together with at least one species of neurite outgrowth-promoting polypeptide of this invention as described herein, dispersed therein as an active ingredient. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a human patient for therapeutic purposes.

For the sake of simplicity, the active agent of the therapeutic compositions described herein shall be referred to as a "neurite outgrowth-promoting polypeptide". It should be appreciated that this term is intended to encompass a variety of AMIGO polypeptides including fusion proteins, synthetic polypeptides, and fragments of naturally ocurring proteins, as well as derivatives thereof, as described herein. This term also encompass the nucleid acids encoding AMIGO polypeptides including fusion proteins, synthetic polypeptides, and fragments of naturally ocurring proteins, as well as derivatives thereof, as described herein.

The methods can optionally be practiced in combination with contacting the neuronal cells or nerves with other agents capable of promoting neuron survivals growth, differentiation or regeneration.

The discovery that AMIGO proteins described herein can promote neurite outgrowth, provides agents for use in improving nerve regeneration or promoting nerve survival, in treating peripheral nerve injury and spinal cord injury, and in stimulation of growth of endogenous, implanted or transplanted CNS tissue.

The present invention therefore also provides a method of promoting regeneration of an injured or severed nerve or nerve tissue, or promoting neurite outgrowth in neuronal cells under a variety of neurological conditions requiring neuronal cell outgrowth. The method comprises contacting a neuronal cell capable of extending neurites, or an injured or severed nerve, with a cell culture system comprising a substrate containing a neurite outgrowth-promoting polypeptide of this invention in an amount effective to promote neurite outgrowth. The method may be carried out in vitro or in vivo.

The polypeptides and nucleic acids used in the present method can be any of the subject polypeptides described herein.

Any of a variety of mammalian neuronal cells can be treated by the present method in the cell culture system, including neuronal cells from brain, CNS, peripheral nerves and the like. In addition, the cells can be from any of a variety of mammalian species, including human, mouse, chicken, and any other mammalian species, including the agricultural stock and non-domesticated mammals.

In selecting a particular subject polypeptide for use in the methods, any of the polypeptides described herein can be utilized to promote neurite outgrowth, irrespective of the species of neuronal cell and species of AMIGO protein from which a subject polypeptide is derived. However, it is preferred to use a human AMIGO protein to induce neurite outgrowth on a human neuronal cell, and the like species selectivity. Thus, in preferred embodiments, the method uses rat neuronal cells and a polypeptide derived from a rat AMIGO protein, or human neuronal cells and a polypeptide derived from a human AMIGO protein, or mouse neuronal cells and a polypeptide derived from a mouse AMIGO protein, etc.

The neurite outgrowth-promoting composition can be attached to the substrate, can be contacted in the liquid phase or in a collagen gel phase. Depending on the assay system used, the AMIGO protein may promote outgrowth when bound onto the solid surfaces but may inhibit the neuronal outgrowth when provided in liqued phase. The composition may contain the subject polypeptide in the form of a fusion protein as described herein. The method may be practiced using the subject polypeptide in any of the various apparati format described herein.

The invention also provides methods and compositions for identifying agents which modulate the interaction of AMIGO with AMIGO, Epiethelial Growth Factor Receptor or AMIGO ligand (AMIGO ligand may be selected from the group of binding partner, endogenous, exogenous protein or substance capable of binding to AMIGO) and for modulating these interactions. The methods for identifying AMIGO modulators find particular application in commercial drug screens. These methods generally comprise (1) combining an AMIGO polypeptide, an AMIGO, EGFR or ligand polypeptide and a candidate agent under conditions whereby, but for the presence of the agent, the AMIGO and AMIGO/EGFR/AMIGO ligand polypeptides engage in a first interaction, and (2) determining a second interaction of the AMIGO and AMIGO/EGFR/AMIGO ligand polypeptides in the presence of the agent, wherein a difference between the first and second interactions indicates that the agent modulates the interaction of the AMIGO and AMIGO/EGFR/AMIGO ligand polypeptides.

The subject methods of modulating the interaction of AMIGO involve combining an AMIGO polypeptide, an AMIGO/EGFR/AMIGO ligand polypeptide and a modulator

under conditions whereby, but for the presence of the modulator, the AMIGO and AMIGO/EGFR/AMIGO ligand polypeptides engage in a first interaction, whereby the AMIGO and AMIGO/EGFR/AMIGO ligand polypeptides engage in a second interaction different from the first interaction. In a particular embodiment, the modulator is dominant negative form of the AMIGO, EGFR or AMIGO ligand polypeptide.

In one embodiment, the present invention provides AMIGO compounds that bind to epidermal growth factor receptor (EGFR), as well as compositions containing one or a combination of such compounds. The AMIGO compounds preferably inhibit (e.g., block) binding of EGFR ligands, such as EGF and TGF-.alpha., to EGFR or even more preferably inhibit the phosphorylation of EGFR. For example, binding of EGFR ligand to EGFR and/or EGFR phosphorylation can be inhibited by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% and preferably results in the prevention of EGFR-mediated cell signaling.

In one embodiment exemplified herein, AMIGO compounds of the invention are AMIGO DNA constructs having an AMIGO cDNA cloned into a vector. Other AMIGO compounds are also encompassed by the invention, including AMIGO peptides, variants, biologically active fragments, an antigenic fragment of AMIGO, anti-AMIGO antibodies or binding portion thereof and and nucleic acids encoding said polypeptides that have retained their binding and/or EGFR phosphorylation inhibiting characteristics. The antibodies can be whole antibodies or antigen-binding fragments of the antibodies, including Fab, F(ab').sub.2, Fv and chain Fv fragments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B. Cloning of AMIGO as an amphoterin-induced gene in hippocampal neurons. (1A) Analysis of ordered differential display on gel electrophoresis, from which the band corresponding to AMIGO was cut for sequencing (marked with arrow). Lane 1, sample from amphoterin matrix; lane 2, sample from laminin matrix. (1B) AMIGO induction was confirmed by using RT-PCR. Lane 1 contains an RT-PCR reaction from hippocampal neurons on amphoterin and lane 2 on laminin. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was analysed as a control.

Figures 2A and 2B. Primary structure of human AMIGO, AMIGO2 and AMIGO3. (2A) Alingment of the three AMIGOs where the identical amino acids between the all AMIGOs are highlighted in red with white letters and similar amino acids are highlighted in red with black letters. Different domains found in the AMIGOs are marked with coloured boxes above the sequences. (2B) Schematic view of the three AMIGOs.

Figure 3. RT-PCR mRNA analysis of AMIGO, AMIGO2 and AMIGO3 in different adult mouse tissues. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Figures 4A, 4B and 4C. In situ hybridization analysis of AMIGO mRNA. In E13 mouse embryo the AMIGO mRNA expression is highest in the dorsal root ganglia (DRG in 4A and 4B) and in the trigeminal ganglion (TG in 4A). (4C) In the adult mouse cerebrum the AMIGO expression is highest in the hippocampal formation where the most intense signal is seen in the dentate gyrus (DG). The pyramidal cell layers CA1-CA3 also express AMIGO.

Figures 5A and 5B. Characterization of the recombinant AMIGO Ig-fusion protein and of anti-AMIGO antibodies. (5A) The recombinant AMIGO Ig-fusion protein (lanes 1, 3 and 5) and protein lysates from adult brain (lanes 2, 4 and 6) were silver stained (lanes 1 and 2) or immunoblotted with rabbit anti-AMIGO antibodies (lanes 3-6). Anti-AMIGO identifies both the AMIGO Ig-fusion protein (lane 3) and one 65 kD band in rat brain lysate (lane 4). Binding of the antibodies to the band corresponding to AMIGO was inhibited by the peptide used for immunization (5A, lanes 5 and 6). Tissue sections were also inhibited in a dose-dependent manner by the peptide (shown for the immunohistochemistry of the adult cerebellum in 5B).

Figure 6. AMIGO expression displays a dual character during brain development. Western blotting of AMIGO using crude brain extracts from different developmental stages reveals clear AMIGO expression during the late embryonic (E) and perinatal development, starting at the E14 stage. The AMIGO expression is downregulated during postnatal (P) stages P6 – P10. The expression is again upregulated between the stages P10 and P12 and remains high in the adult brain. The upregulation coincides with the onset of myelination as

demonstrated by the comparison with the CNPase expression. The expression of AMIGO and CNPase display a parallel increase during postnatal development. W; postnatal week.

Figures 7A, 7B, 7C, 7D, 7E and 7F. AMIGO is localized to axonal fiber tracts in tissue and in cultured cells. Immunohistochemical staining of rat tissues revealed that AMIGO is specifically expressed in the nervous system. In E15 embryo (7A) immunostaining is observed in developing fiber tracts and nerves, like in the ventral part of the marginal layer (ml) of the spinal cord (SC) and in the nerves connecting to the dorsal root ganglion (DRG) and to the spinal cord. In the adult animal (7B, 7C, 7D and 7E) AMIGO is also detected in nerve fibers. In the cerebellum (7B) the most intense staining is detected in fibers on both sides of the granule cell layer (G), as in the characteristic basket-like structure (arrow) formed by the basket cell axons around the Purkinje cell soma (p). Fibers in the cerebellar white matter (W) are also stained. In general, myelinated fiber tracts are clearly stained in adult animals as demonstrated by the similar staining of AMIGO (panel 7C) and CNPase (panel 7D) around the hippocampus in sagittal sections. In addition to the cerebellar basket cell axons, non-myelinated fibers are also stained in hippocampus (panel 7C, higher magnification in panel 7E). These CNPase negative fibers of the hippocampus reside in the vicinity of the CA3 pyramidal cell bodies. In cultured hippocampal neurons (panel 7F) AMIGO is also detected in neuronal processes by immunofluorescence staining. G, granule cell layer of the cerebellar cortex; M; molecular layer of the cerebellar cortex; CA1, CA1 region; CA3, CA3 region; h, hilus. Scale bar 50 μm in panels 7A, 7B, 7E and 7F; 500 μm in panels 7C and 7D.

Figures 8A, 8B, 8C and 8D. AMIGO promotes neurite outgrowth of hippocampal neurons.

(8A) Substratum coated with 25 μg/ml of AMIGO promotes neurite outgrowth of E18 hippocampal neurons. (8B) Cells on the control substratum coated with 25 μg/ml of the Fc protein without the AMIGO ectodomain is shown for comparison. (8C) AMIGO induced hippocampal neurite outgrowth after 24 h of culture. The AMIGO Ig-fusion (gray bars) and the Fc control protein (black bars) were coated as follows; 0 μg/ml (1), 3.125 μg/ml (2), 6.25 μg/ml (3), 12.5 μg/ml (4), 25 μg/ml (5), 50 μg/ml (6), 100 μg/ml (7). (8D) AMIGO-induced neurite outgrowth (the substratum coated with 25μg/ml of the AMIGO Ig-fusion protein) is blocked by the AMIGO Ig-fusion protein in the assay medium. The

AMIGO Ig-fusion (gray bars) and the Fc control protein (black bars) were added into the culture medium as follows; 0 μg/ml (1), 3.125 μg/ml (2), 6.25 μg/ml (3), 12.5 μg/ml (4), 25 μg/ml (5), 50 μg/ml (6), 100 μg/ml (7). The error bars give the standard deviation calculated from 15 microscopy fields in three independent experiments. Scale bar 50 μm in panels 8A and 8B.

Figures 9A, 9B, and 9C. Soluble AMIGO inhibits fasciculation in hippocampal neurons. (9A) E18 hippocampal neurons on poly-L-lysine substratum with 25 μg/ml of the AMIGO Ig-fusion in the culture medium. (9B) E18 hippocampal neurons on poly-L-lysine substratum with 25 μg/ml the Fc control protein is shown for comparison. (9C) Total length of processes, the diameter of which is < 2μm (formed from 1-3 neurites) on poly-L-lysine substratum. The AMIGO Ig-fusion and (gray bars) and the Fc control protein (black bars) were added into the culture medium as follows; 0 μg/ml (white bar)(1), 3.125 μg/ml (2), 6.25 μg/ml (3), 12.5 μg/ml (4), 25 μg/ml (5). The error bars in panel 9C give the standard deviation calculated from 12 microscopy fields in three independent experiments. Scale bar 50 μm in panel 9A and 9B.

Figures 10A, 10B, 10C, and 10D. Homophilic interaction of AMIGO. (10A) Co-immunoprecipitation experiment. Lane 1, cells transfected with full-length GFP-tagged AMIGO and full-length V5-tagged AMIGO; lane 2, full length GFP-tagged AMIGO and soluble V5-tagged AMIGO; lane 3, transfected only with full-length GFP-tagged AMIGO; lane 4, full length GFP-tagged AMIGO and full-length V5-tagged human RAGE. Full length GFP-tagged AMIGO was co-immunoprecipitated with full length V5-tagged AMIGO (lane 1) and by using soluble V5-tagged AMIGO containing only the ectodomain (lane 2) Co-immunoprecipitation could be also shown by precipitating with GFP antibody. (10B) Kinetics of bead aggregation. N_t and N_0 are the total number of particles at incubation times t and 0 respectively. The extent of bead aggregation is represented by the index N_t/ N_0. Gray bars represent AMIGO Ig-fusion coated beads and black bars Fc coated beads. (10C-10D) Bead aggregation after 60 min using protein A beads coated with the AMIGO Ig-fusion (10C) or with the Fc control (10D). The error bars give the standard deviation calculated from 12 microscopy fields in three independent experiments.

Figure 11. Multiple alignment for the leucine-rich repeat areas of Slit1, Nogo-receptor and AMIGO. The identical amino acids between Slit1 and Nogo-receptor compared to the AMIGO are highlighted in black and similar amino acids are highlighted in gray. The consensus sequence of the 6 LRR motifs of the AMIGO are shown above the sequences.

Figure 12. The three dimensional structure of the immunoglobulin domain, schematic presentation. Ig-domain is a sandwich of two antiparallel beta sheets. (Principles of Biochemistry, Horton et al. 2002)

Figures 13A and 13B. Structure of ribonuclease inhibitor. 13A) Ribbon diagram of the structure of porcine RI generated using the program MOLSCRIPT. 13B) Consensus sequences and secondary structure of LRRs of porcine RI. The sequence of RI was aligned so that two types of repeats (A and B) alternate in the sequence. One-letter amino acid code is used. 'x' indicates any amino acid and 'a' denotes an aliphatic amino acid. The part of the repeat that is strongly conserved in all LRR proteins is underlined, and the conserved residues are shown in bold. Below the sequence, solid lines mark the core region of Ig-sheet and helix; dots denote extensions of helix in different repeats. (Kobe and Deisenhofer 1995)

Figure 14. Structure of RNase A-RI complex. In the ribbon diagram, RNase A is dark and RI is light. (Kobe and Deisenhofer 1995)

Figure 15. Schematic drawings of the structures of some LRR-containing proteins. Tartan is a protein involved in *Drosophila* development (Chang et al. 1993, Milan et al. 2001). Slit protein contains additional domains, which are not represented here. Sig, signal peptide; AFR, amino terminal flanking region; LRR, leucine-rich repeat; CFR, carboxy terminal flanking region; PI, phosphatidylinositol. (Hayata et al. 1998)

Figure 16. Schematic presentation of the predicted structure of AMIGO.

Figure 17. Specificity of AMIGO staining in tissue. α-AMI (anti-AMIGO antibody) was incubated with rising concentrations of AMIP2 or AMIP1 peptide. Tissue sections from rat cerebellum were stained with peptide incubated antibody. Increasing concentration of AMIP2 peptide decreases and finally blocks the tissue staining completely. Evidently, the

binding of α-AMI to tissue sections is inhibited by AMIP2 peptide. Control peptide AMIP1 does not have an effect on α-AMI binding even in high concentrations.

Figure 18. Coronal section of the rat cerebrum stained with α-AMI. Myelinated fiber tracts are clearly stained. Some areas of the cerebral cortex are stained and one of them is marked with arrowhead (same areas are also stained with the oligodendrocyte marker α-CNPase and with α-NF-M). Non-myelinated structures are stained in the CA3 region of the hippocampus (arrow).

Figures 19A and 19B. Coronal section of the hippocampal CA3 region (arrow in figure 7, higher magnification). 19A) staining with α-AMI 19B) staining with α-NF-M. Both stainings are located near the proximal part of the apical dendrites of the pyramidal cells. This layer is called stratum lucidum. Less intensively stained round structures are the cell nuclei stained with hematoxylin. Scale bar 25 μm.

Figures 20A, 20B, 20C, and 20D. Sagittal sections of the rat hippocampus. 20a) staining with α-AMI 20b) staining with α-CNPase. Both antibodies stain the myelinated nerve fibers in and around the hippocampus. In addition, α-AMI causes strong staining of non-myelinated structures in the CA3 region of the hippocampus and in the hilus (h) of the dentate gyrus (DG). 20c) and 20d) Higher magnification of staining with α-AMI. The staining of the CA3 region and of the dentate gyrus has a fiber-like structure (arrows). Scale bar 50 μm.

Figures 21A and 21B. Higher magnification of the cerebral cortex. 21a) staining with α-AMI 21b) staining with α-NF-M. Same areas of the cerebral cortex are stained both with α-AMI and with α-NF-M. In closer examination, some of the thick apical dendrites of the pyramidal cells (**arrows**) and some thin fibers (**arrowheads**) are stained with both antibodies. Cell soma and basal dendrites of pyramidal cells are also stained with α-NF-M. Scale bar 50 μm.

Figures 22A and 22B. Cerebellar sections stained with α-AMI. 22a) Coronal section of the cerebellum. Cerebellar cortex consists of three layers: outermost molecular layer (**M**), Purkinje cell layer (**P**) and granule cell layer (**G**). White matter (**W**) underlies the

cerebellar cortex. α-AMI appears to stain myelinated fibers in the white matter and in the granule cell layer. The basket-like structure (**arrow**) formed by the basket cell axons around the Purkinje cell soma (**p**) and fibers in the molecular layer are also stained. In the molecular layer the staining is restricted to the inner part of the layer and stained fibers mainly run parallel to Purkinje cell layer. 22b) Sagittal section of the cerebellum. In the medial part of the cerbellum, the structure of the staining in white matter (**W**) resembles a string of pearls. Scale bar 25 μm.

Figures 23A, 23B, and 23C. Transverse section of the spinal cord white matter. 23a) staining with α-AMI 23b) staining with α-CNPase 23c) staining with α-NF-M. Clear, round areas of the section are the myelin sheaths. Small spots (**arrow**) are clearly stained inside the myelin sheath with α-AMI and with α-NF-M. These spots seem to be the transections of axons. They are not stained with α-CNPase. The axon tracts running parallel to the section plane are stained with α-NF-M but not with α-AMI. Scale bar 50 μm.

Figures 24A and 24B. Immunohistochemistry of the kidney. Staining with α-AMI (24a) or with α-NF-M (24b) detects the same small structures in the kidney (**arrows**). Consequently, α-AMI staining is located in the nerves of the kidney. Scale bar 100 μm.

Figures 25A, 25B, 25C, and 25D. Immunohistochemistry of the head of 18 day old embryo. Staining with α-AMI detects developing fiber tracts and cranial nerves, like the optic nerve (in panel 25a) and the internal capsule (in panel 25c). Staining in retina (arrow in panel 25a) is located in the nerve fiber layer. Nerve fiber layer consist of ganglion cell axons, which form the optic nerve. Control sections (panel 25b and 25d) are stained with AMIP2 blocked α-AMI. Scale bar 100 μm.

Figures 26A and 26B. Western blotting of AMIGO using crude rat brain extracts from different developmental stages. Same total weight of tissue was used from each sample. Brains of 16- and 18-day old embryo (E16 and E18), of 1-, 2-, 4-, 6-, 8-, 10- and 14-day old rat (P1-P14) and of adult rat were used. AMIGOIg fusion protein (AMIIg) was used as a control sample. In panel 26a) Western blot is detected with α-AMI and α-CNPase. α-

AMI detects about 65 kDa protein band and weaker protein band, about 130 kDa. α-CNPase detects about 48 kDa protein band. The AMIGO expression displays dual character during brain development. Immunoblotting reveals clear AMIGO expression during the late embryonic (E) and perinatal development. The AMIGO expression is downregulated during postnatal (P) stages P4 – P10. The expression is again upregulated between stages P10 and P14 and remains high in the adult brain. The upregulation coincides with the onset of myelination as demonstrated by the comparison with the CNPase expression. The expression of AMIGO and CNPase display a parallel increase during postnatal development. In panel 26b) Western blot is stained with Ponceau stain to compare the total amount of protein in each sample.

Figure 27. Coimmunoprecipitation of AMIGOs with EGFR. Stable EGFR expressing 293 cells were transfected with V5-tagged full length AMIGO (lane1), EC-part containing AMIGO (lane 2), full length AMIGO2 (lane 3) or with full length AMIGO3 (lane 4). The coimmunoprecipitations were done by using anti-EGFR antibodies and the detection was done by using anti-V5 antibodies. The result shows that both AMIGO and AMIGO2 bind the EGFR and only the EC-part is enough for the binding (shown for the AMIGO).

Figure 28. Homo- and heterophilic binding of AMIGO, AMIGO2 and AMIGO3. Coimmunoprecipitation was done by using anti-V5-tag antibodies and the detection was done by using anti-GFP-tag antibodies. Lanes 1-5 contains full length AMIGO with GFP-tag; lanes 6-9 contains full length AMIGO2 with GFP-tag; lanes 10-12 full length AMIGO3 with GFP-tag. V5-tagged proteins used in this experiment: AMIGO full length in lane 1; AMIGO EC-part in lane 2; AMIGO2 full length in lanes 3 and 6; AMIGO2 EC-part in lane 7; AMIGO3 full length in lanes 4, 8 and 10; AMIGO3 EC-part in lane 11; RAGE full length in lanes 5, 9 and 12. Pictures shows that full length AMIGO-GFP could be co-immunoprecipitated with full length AMIGO, AMIGO2 and AMIGO3 but not with full length RAGE. Full length AMIGO-GFP could also be coimmunoprecipitated with only EC-part containing AMIGO. The full length AMIGO2-GFP could be coimmunoprecipitated with full length AMIGO2 and AMIGO3 but also with only EC-part containing AMIGO2. The full length AMIGO3-GFP could be coimmunoprecipitated with full length AMIGO3 and only EC-part containing AMIGO3. The coimmunoprecipitation results show that AMIGOs could bind each others in heterophilically but they also posses homophilic binding properties.

Figure 29. AMIGO inhibits EGFR phosphorylation. When AMIGO and flag-tagged human EGFR are expressed together in HEK293T cells AMIGO clearly inhibits the EGFR autophosphorylation induced by EGF ligation when compared to AMIGO2, AMIGO3 and vector control.

DETAILED DESCRIPTION OF THE INVENTION

Amphoterin and laminin are both neurite outgrowth-promoting factors. The genes induced on amphoterin matrix were detected by using the ordered differential display method (Matz et al., 1997) from hippocampal neurons, which were cultured on amphoterin or laminin matrix. A novel gene was seen to be induced on amphoterin. The whole coding sequence for this differentially expressed gene was cloned and named as *AMIGO* (*AMphoterin Induced Gene and Orphan receptor*). The predicted amino acid sequences of the AMIGO codes type I transmembrane protein containing a signal sequence for secretion and a transmembrane domain. The deduced extracellular part of the AMIGO contains six leucine-rich repeats (LRRs) flanked by cysteine-rich LRRN- and LRRC-terminal domains and one immunoglobulin domain close to the transmembrane region. The deduced 100 amino acid long cytosolic part of the protein do not contain any known domains.We have also identified a novel family of transmembrane proteins consisting of AMIGO, AMIGO2 and AMIGO3. These three proteins show clear homology with each other; their length and location of different domains are highly identical (Fig. 2 B).

Based on RT-PCR experiments, in situ hybridization and immunohistochemistry, AMIGO is an essentially nervous system specific protein. One cellular mechanism in the growth of axonal connections is fasciculation: axons grow along each other by using pioneer axons as the substratum for the growth cones of the later ones. Interestingly, a dominant negative approach using AMIGO ectodomain in the culture medium clearly suggests a role for AMIGO in fasciculation. Further, AMIGO displays a homophilic binding mechanism that would explain its role in fasciculation. It is also noteworthy that the LRR sequences of the AMIGOs display homology with the slit proteins and with the Nogo receptor (Fig. 11) that have been implicated in axon growth, regeneration and guidance. The second upregulation of the AMIGO expression suggests a role in myelination. It seems reasonable that AMIGO would mediate cell-to-cell interactions also at this stage of development. Further, AMIGO

expression remains high until adulthood. This suggests that AMIGO plays a role in regeneration and plasticity of the adult fiber tracts, the mechanisms of which commonly recapitulate mechanisms of fiber tract development.

Thus, this invention is based on the discovery and characterization of a novel human gene/protein termed AMIGO and its homologous counterparts designated as AMIGO2 and AMIGO3. Together these three proteins form a novel family of transmembrane proteins (for simplicity, all of these proteins are hereinafter referred as AMIGO or AMIGOs).

In one embodiment, the invention provides a purified protein comprising, or alternatively consisting of a polypeptide, a biologically active fragment, or an antigenic fragment of AMIGO.

In another embodiment the present invention is directed to proteins which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to AMIGO protein.

Due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic acid sequence of the cDNA contained in SEQ ID NO:1, 3, or 5 or fragments thereof, will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g. replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino

acid sequence to be changed.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

In addition to naturally occurring allelic variants of AMIGO, changes can be introduced by mutation into AMIGO sequences that incur alterations in the amino acid sequences of the encoded AMIGO polypeptide. Nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of an AMIGO polypeptide. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of AMIGO without altering its biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the AMIGO molecules of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well known in the art. Useful conservative substitutions are shown in Table B, "Preferred substitutions." Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) could be used. See Cunningham et al., Science 244:1081-1085 (1989). The resulting mutant molecules can then be tested for biological activity. Besides conservative amino acid substitutions (See Table B below), variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitutions with one or more of the amino acid residues

having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example,896I polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion peptide, serum albumin (preferably human serum albumin) or a fragment or variant thereof, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

Table B. Preferred substitutions

Original residue	Exemplary substitutions	Preferred substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, Norleucine	Leu

A further embodiment of the invention relates to polypeptides which comprise the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more

preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions from a polypeptide sequence disclosed herein. It is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide, a portion, or a complement of SEQ ID NO:2, 4 or 6 in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4,3, 2 or 1 amino acid substitutions.

In preferred embodiments, the amino acid substitutions are conservative.

In specific embodiments, the polypeptides of the invention comprise, or alternatively, consist of, fragments or variants of a reference amino acid sequence encoded by SEQ ID NO:2, 4 or 6 wherein the fragments or variants have 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, amino acid residue additions, substitutions, and/or deletions when compared to the reference amino acid sequence.

In one embodiment techniques suitable for the production of AMIGO polypeptide are well known in the art and include isolating AMIGO from an endogenous source of the polypeptide, peptide synthesis (using a peptide synthesizer) and recombinant techniques (or any combination of these techniques).

In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45%, preferably 60%, more preferably 70%, 80%, 90%, and most preferably about 95% homologous to that of an AMIGO.

In another embodiment, AMIGO polypeptide variants have at least (1) about 80% amino acid sequence identity with a full-length native AMIGO polypeptide sequence shown in SEQ ID NO:2, 4 or 6 (2) an AMIGO polypeptide sequence lacking the signal peptide, (3) any other fragment of a full-length AMIGO polypeptide sequence. For example, AMIGO polypeptide variants include AMIGO polypeptides wherein one or more amino acid residues are added or deleted at the N- or C-terminus of the full-length native amino acid sequence. An AMIGO polypeptide variant will have at least about 80% amino acid sequence identity, preferably at least about 81% amino acid sequence identity, more

preferably at least about 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% amino acid sequence identity and most preferably at least about 99% amino acid sequence identity with a full-length native sequence AMIGO polypeptide sequence. An AMIGO polypeptide variant may have a sequence lacking the signal peptide or any other fragment of a full-length AMIGO polypeptide sequence. Ordinarily, AMIGO variant polypeptides are at least about 10 amino acids in length, often at least about 20 amino acids in length, more often at least about 30, 40, 50, 60, 70, 80, 90, 100 or150 amino acids in length, or more.

One aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of (a) a nucleotide sequence described in SEQ ID NO:1, 3 or 5 (b) a nucleotide sequence in SEQ ID NO:1, 3 or 5 part of which encodes a mature AMIGO polypeptide; (c) a nucleotide sequence which encodes a biologically active fragment of an AMIGO polypeptide; (d) a nucleotide sequence which encodes an antigenic fragment of an AMIGO polypeptide; (e) a nucleotide sequence complementary to any of the nucleotide sequences in (a),(b), (c), (d), above.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences due to degeneracy of the genetic code and thus encode the same AMIGO protein as shown in sequence of SEQ ID NO:2, 4 or 6.

In addition sequence polymorphisms that change the amino acid sequences of the AMIGO may exist within a population. For example, allelic variation among individuals will exhibit genetic polymorphism in AMIGO. The terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding AMIGO, preferably a vertebrate AMIGO. Such natural allelic variations can typically result in 1-5% variance in AMIGO. Any and all such nucleotide variations and resulting amino acid polymorphisms in the AMIGO, which are the result of natural allelic variation and that do not alter the functional activity of the AMIGO are within the scope of the invention.

Moreover, AMIGO from other species that have a nucleotide sequence that differs from the human sequence of AMIGO are contemplated. Nucleic acid molecules corresponding to natural allelic variants and homologues of AMIGO cDNAs of the invention can be

isolated based on their homology to AMIGO using cDNA-derived probes to hybridize to homologous AMIGO sequences under stringent conditions.

"AMIGO variant polynucleotide" or "AMIGO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active AMIGO that (1) has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native AMIGO, (2) a full-length native AMIGO lacking the signal peptide, or (3) any other fragment of a full-length AMIGO. Ordinarily, an AMIGO variant polynucleotide will have at least about 80% nucleic acid sequence identity, more preferably at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% nucleic acid sequence identity and yet more preferably at least about 99% nucleic acid sequence identity with the nucleic acid sequence encoding a full-length native AMIGO. An AMIGO variant polynucleotide may encode full-length native AMIGO lacking the signal peptide with or without the signal sequence, or any other fragment of a full-length AMIGO. Variants do not encompass the native nucleotide sequence.

Ordinarily, AMIGO variant polynucleotides are at least about 30 nucleotides in length, often at least about 60, 90, 120, 150, 180, 210, 240, 270, 300, 400 nucleotides in length, more often at least about 500 nucleotides in length, or more.

The structure and sequence of the mammalian AMIGO cDNA sequence which encodes the mouse and human sequences disclosed herein, make it possible to clone gene sequences from other mammals which encode the AMIGO. Of particular interest to the present invention is the ability to clone the human AMIGO molecules using the sequences disclosed herein. The DNA encoding AMIGO may be obtained from any cDNA library prepared from tissue believed to possess the AMIGO mRNA and to express it at a detectable level, as shown herein in the Examples. Accordingly, AMIGO DNA can be conveniently obtained from a cDNA library prepared, for example, from mammalian fetal liver, brain, muscle, intestine, and peripheral nerves. The AMIGO-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries are screened with probes (such as antibodies to the AMIGO or oligonucleotides of about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using

standard procedures as described in chapters 10-12 of Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989) or alternatively to use PCR methodology as described in section 14 of Sambrook et al., supra.

Amino acid sequence variants of AMIGO are prepared by introducing appropriate nucleotide changes into the AMIGO DNA, or by synthesis of the desired AMIGO polypeptide. Such variants represent insertions, substitutions, and/or specified deletions of, residues within or at one or both of the ends of the amino acid sequence of a naturally occurring AMIGO with sequence of SEQ ID NO:2, 4 or 6. Preferably, these variants represent insertions and/or substitutions within or at one or both ends of the mature sequence, and/or insertions, substitutions and/or specified deletions within or at one or both of the ends of the signal sequence of the AMIGO. Any combination of insertion, substitution, and/or specified deletion is made to arrive at the final construct, provided that the final construct possesses the desired biological activity as defined herein.

Variations in the native sequence as described above can be made using any of the techniques and guidelines for conservative and non-conservative mutations set forth in U.S. Pat. No. 5,364,934. These include oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis.

The nucleic acid (e.g., cDNA or genomic DNA) encoding the AMIGO is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

The AMIGOs of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. Fusion proteins can be easily created using recombinant methods. A nucleic acid encoding AMIGO can be fused in-frame with a non-AMIGO encoding nucleic acid, to the AMIGO N- or COOH-terminus, or internally. Fusion genes may also be synthesized by conventional techniques, including automated DNA synthesizers. An AMIGO fusion protein may include any portion to the entire AMIGO,

including any number of the biologically active portions. Fusion polypeptides are useful in expression studies, cell-localization, bioassays, and AMIGO purification

Alternatively, AMIGO fusion protein can also be easily created using PCR amplification and anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (Ausubel et al., supra).

The signal sequence may be a component of the vector, or it may be a part of the AMIGO DNA that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native AMIGO signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, e.g., the yeast invertase leader, alpha-factor leader (including *Saccharomyces* and *Kluyveromyces*, alpha-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued Apr. 23, 1991), or acid phosphatase leader, the *Candida albicans* glucoamylase leader (EP 362,179 published Apr. 4, 1990). In mammalian cell expression the native signal sequence (e.g., the AMIGO presequence that normally directs secretion of AMIGO from human cells in vivo) is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from other animal AMIGOs, and signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal.

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the AMIGO nucleic acid. Vector choice is dictated by the organism or cells being used and the desired fate of the vector. Vectors may replicate once in the target cells, or may be "suicide" vectors. In general, vectors comprise signal sequences, origins of replication, marker genes, enhancer elements, promoters, and transcription termination sequences. The choice of these elements depends on the organisms in which the vector will be used and are easily determined. Some of these elements may be conditional, such as an inducible or conditional promoter that is turned

"on" when conditions are appropriate.

Vectors can be divided into two general classes: Cloning vectors are replicating plasmid or phage with regions that are non-essential for propagation in an appropriate host cell, and into which foreign DNA can be inserted; the foreign DNA is replicated and propagated as if it were a component of the vector. An expression vector (such as a plasmid, yeast, or animal virus genome) is used to introduce foreign genetic material into a host cell or tissue in order to transcribe and translate the foreign DNA. In expression vectors, the introduced DNA is operably linked to elements, such as promoters, that signal to the host cell to transcribe the inserted DNA. Some promoters are exceptionally useful, such as inducible promoters that control gene transcription in response to specific factors. Operably linking AMIGO or anti-sense construct to an inducible promoter can control the expression of AMIGO or fragments, or anti-sense constructs. Examples of classic inducible promoters include those that are responsive to a-interferon, heat-shock, heavy metal ions, and steroids such as glucocorticoids (Kaufman RJ, Vectors Used for Expression in Mammalian Cells," Methods in Enzymology, Gene Expression Technology, David V. Goeddel, ed., 1990, 185:487-511) and tetracycline. Other desirable inducible promoters include those that are not endogenous to the cells in which the construct is being introduced, but, however, is responsive in those cells when the induction agent is exogenously supplied.

Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence, such as the AMIGO nucleic acid sequence, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to AMIGO-encoding DNA by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native AMIGO promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the AMIGO DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of AMIGO as compared to the native AMIGO

promoter. Various promoters exist for use with prokaryotic, eukaryotic, yeast and mammalian host cells, known for skilled artisan.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding AMIGO.

Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding AMIGO. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector, Sambrook et al., supra, pp. 16.17 - 16.22. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of AMIGO that are biologically active.

Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. See, e.g., Tissue Culture, Academic Press, Kruse and Patterson, editors (1973). Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci USA, 77:4216 (1980)); human cervical carcinoma cells (HELA, ATCC CCL 2); and canine kidney cells (MDCK, ATCC CCL 34);

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors for AMIGO production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook et al., supra, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers.

General aspects of mammalian cell host system transformations have been described in U.S. Pat. No. 4,399,216 issued Aug. 16, 1983. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. USA, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, etc., may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Prokaryotic cells used to produce the AMIGO polypeptide of this invention are cultured in suitable media as described generally in Sambrook et al., supra. In general, principles, protocols, and practical techniques for maximizing the productivity of mammalian cell cultures can be found in Mammalian Cell Biotechnology: a Practical Approach, M. Butler, ed. (IRL Press, 1991).

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 (1980)), dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide or antibodies recognizing specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression, alternatively, can be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu et al., Am. J. Clin. Path., 75:734-738 (1980).

RECOMBINANT PRODUCTION

When AMIGO is produced in a recombinant cell other than one of human origin, the AMIGO is completely free of proteins or polypeptides of human origin. However, it is necessary to purify AMIGO from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to AMIGO. As a first step, the culture medium or lysate can be centrifuged to remove particulate cell debris. AMIGO can then be purified from contaminant soluble proteins and polypeptides with the following procedures, which are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica; chromatofocusing; immunoaffinity; epitope-tag binding resin; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG.

AMIGO variants in which residues have been deleted, inserted, or substituted are recovered in the same fashion as native sequence AMIGO, taking account of any substantial changes in properties occasioned by the variation. Immunoaffinity resins, such as a monoclonal anti-AMIGO resin, can be employed to absorb the AMIGO variant by binding it to at least one remaining epitope.

Variants can be assayed as taught herein. A change in the immunological character of the AMIGO molecule, such as affinity for a given antibody, can be measured by a competitive-type immunoassay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

This invention encompasses chimeric polypeptides comprising AMIGO fused to a heterologous polypeptide. A chimeric AMIGO is one type of AMIGO variant as defined herein. In one preferred embodiment, the chimeric polypeptide comprises a fusion of the AMIGO with a tag polypeptide which provides an epitope to which an anti-tag antibody or molecule can selectively bind. The epitope-tag is generally provided at the amino- or carboxyl- terminus of the AMIGO. Such epitope-tagged forms of the AMIGO are desirable, as the presence thereof can be detected using a labeled antibody against the tag polypeptide. Also, provision of the epitope tag enables the AMIGO to be readily purified by affinity purification using the anti-tag antibody. Affinity purification techniques and diagnostic assays involving antibodies are described later herein.

Tag polypeptides and their respective antibodies are well known in the art. Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky et al., Protein Engineering, 3(6):547-553 (1990)). Other tag polypeptides have been disclosed. Examples include the Flag-peptide (Hopp et al., BioTechnology, 6:1204-1210 (1988)); the KT3 epitope peptide (Martin et al., Science, 255:192-194 (1992)); an alpha-tubulin epitope peptide (Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)); and the T7 gene protein peptide tag (Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)).

Once the tag polypeptide has been selected, an antibody thereto can be generated using the techniques disclosed herein. A C-terminal poly-histidine sequence tag is preferred. Poly-histidine sequences allow isolation of the tagged protein by Ni-NTA chromatography as described (Lindsay et al. Neuron 17:571-574 (1996)), for example.

The general methods suitable for the construction and production of epitope-tagged AMIGO are the same as those disclosed hereinabove.

Epitope-tagged AMIGO can be conveniently purified by affinity chromatography using the anti-tag antibody. The matrix to which the affinity antibody is attached is most often agarose, but other matrices are available (e.g. controlled pore glass or poly(styrenedivinyl)benzene). The epitope-tagged AMIGO can be eluted from the affinity column by varying the buffer pH or ionic strength or adding chaotropic agents, for example.

Chimeras constructed from an AMIGO sequence linked to an appropriate immunoglobulin constant domain sequence (immunoadhesins) are known in the art. Immunoadhesins reported in the literature include fusions of the T cell receptor (Gascoigne et al., Proc. Natl. Acad. Sci. USA, 84: 2936-2940 (1987)); CD4* (Capon et al., Nature 337: 525-531 (1989); Traunecker et al., Nature, 339: 68-70 (1989); Zettmeissl et al., DNA Cell Biol USA, 9: 347-353 (1990); Byrn et al., Nature, 344: 667-670 (1990)); TNF receptor (Ashkenazi et al., Proc. Natl. Acad. Sci. USA, 88: 10535-10539 (1991); Lesslauer et al., Eur. J. Immunol., 27: 2883-2886 (1991); Peppel et al., J. Exp. Med., 174:1483-1489 (1991)); and IgE receptor alpha* (Ridgway et al., J. Cell. Biol., 1 15:abstr. 1448 (1991)), where the asterisk (*) indicates that the receptor is member of the immunoglobulin superfamily.

The simplest and most straightforward immunoadhesin design combines the binding region(s) of the "adhesin" protein with the hinge and Fc regions of an immunoglobulin heavy chain. Ordinarily, when preparing the AMIGO-immunoglobulin chimeras of the present invention, nucleic acid encoding the AMIGO will be fused C-terminally to nucleic acid encoding the N-terminus of an immunoglobulin constant domain sequence, however N-terminal fusions are also possible.

Typically, in such fusions the encoded chimeric polypeptide will retain at least functionally

active hinge and CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the CH1 of the heavy chain or the corresponding region of the light chain.

The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimise the biological activity, secretion or binding characteristics of the AMIGO-immunoglobulin chimeras.

The choice of host cell line for the expression of AMIGO immunoadhesins depends mainly on the expression vector. Another consideration is the amount of protein that is required. Milligram quantities often can be produced by transient transfections utilizing, for example, calcium phosphate or DEAE-dextran method (Aruffo et al., Cell, 61:1303-1313 (1990); Zettmeissl et al., DNA Cell Biol. US, 9:347-353 (1990)). If larger amounts of protein are desired, the immunoadhesin can be expressed after stable transfection of a host cell line, for example, introducing the expression vectors into Chinese hamster ovary (CHO) cells in the presence of an additional plasmid encoding dihydrofolate reductase.

ANTIBODIES

AMIGO nucleic acid is useful for the preparation of AMIGO polypeptide by recombinant techniques exemplified herein which can then be used for production of anti-AMIGO antibodies having various utilities described below.

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal.

The invention further includes an antibody that specifically binds with AMIGO, or a fragment thereof. In a preferred embodiment, the invention includes an antibody that inhibits the biological activity of AMIGO. The antibody is useful for the identification for AMIGO in a diagnostic assay for the determination of the levels of AMIGO in a mammal having a disease associated with AMIGO levels. In addition, an antibody that specifically binds AMIGO is useful for blocking the interaction between AMIGO and its receptor, and

is therefore useful in a therapeutic setting for treatment of AMIGO related disease, as described herein.

Monoclonal antibodies directed against full length or peptide fragments of an AMIGO protein or peptide may be prepared using any well-known monoclonal antibody preparation procedures, such as those described, for example, in Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY). Anti-AMIGO mAbs may be prepared using hybridoma methods comprising at least four steps: (1) immunizing a host, or lymphocytes from a host; (2) harvesting the mAb secreting (or potentially secreting) lymphocytes, (3) fusing the lymphocytes to immortalized cells, and (4) selecting those cells that secrete the desired (anti-AMIGO) mAb. The mAbs may be isolated or purified from the culture medium or ascites fluid by conventional Ig purification procedures such as protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, ammonium sulfate precipitation or affinity chromatography (Harlow et al, supra).

A mouse, rat, guinea pig, hamster, or other appropriate host is immunized to elicit lymphocytes that produce or are capable of producing Abs that will specifically bind to the immunogen. Alternatively, the lymphocytes may be immunized in vitro.

If human cells are desired, peripheral blood lymphocytes are generally used; however, spleen cells or lymphocytes from other mammalian sources are preferred.

The immunogen typically includes AMIGO or an AMIGO fusion protein.

The invention further comprises humanized and human anti-AMIGO Abs.

Humanized forms of non-human Abs are chimeric Igs, Ig chains or fragments (such as Fv, Fab, Fab', F(ab') or other antigen-binding subsequences of Abs) that contain minimal sequence derived from non-human Ig.

Generally, a humanized antibody has one or more amino acid residues introduced from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization is accomplished by substituting rodent CDRs or CDR sequences for the corresponding

sequences of a human antibody (Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science 239:1534-1536, (1988). Such "humanized" Abs are chimeric Abs (U. S. Patent No. 4816567, 1989), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized Abs are typically human Abs in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent Abs. Humanized Abs include human Igs (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit, having the desired specificity, affinity and capacity. In some instances, corresponding non-human residues replace Fv framework residues of the human Ig. Humanized Abs may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody comprises substantially all of at least one, and typically two, variable domains, in which most if not all of the CDR regions correspond to those of a non-human Ig and most if not all of the FR regions are those of a human Ig consensus sequence. The humanized antibody optimally also comprises at least a portion of an Ig constant region typically that of a human Ig (Jones et al., supra; Presta LG, Curr Opin Biotechnol 3:394-398 (1992).

Human Abs can also be produced using various techniques, including phage display libraries (Hoogenboom et al., Nucleic Acids Res 19:4133-4137 (1991); Marks et al., Biotechnology (NY) 10:779-83 (1991) and the preparation of human mAbs (Boerner et al., J Immunol 147(1):86-95 (1991); Reisfeld and Sell, Monoclonal Antibodies and Cancer Therapy Alan R. Liss, Inc., New York (1985). Similarly, introducing human Ig genes into transgenic animals in which the endogenous Ig genes have been partially or completely inactivated can be exploited to synthesize human Abs. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire (U. S. Patent No. 5545807, 1996; U. S. Patent No. 5545806, 1996 ; U. S. Patent No. 5569825, 1996 ; U. S. Patent No. 5633425, 1997 ; U. S. Patent No. 5661016, 1997 ; U. S. Patent No. 5625126, 1997; Fishwild et al., Nat Biotechnol 14:845-51 (1996); Lonberg and Huszar, Int Rev Immunol 13:65-93 (1995); Lonberg et al., Nature 368:856-9 (1994); Marks et al., Biotechnology (NY) 10:779-783 (1992)).

In one preferred embodiment the instant inventions also comprises bi-specific mAbs that are monoclonal, preferably human or humanized, that have binding specificities for at least two different antigens. For example, a binding specificity is AMIGO; the other is for any antigen of choice, preferably a cell surface protein or receptor or receptor subunit.

Traditionally, the recombinant production of bi-specific Abs is based on the co-expression of two Ig heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537-540 (1983)). Because of the random assortment of Ig heavy and light chains, the resulting hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the desired bi-specific structure. The desired antibody can be purified using affinity chromatography or other techniques (WO 93/08829, (1993); Traunecker et al., Trends Biotechnol 9:109-113 (1991)).

To manufacture a bi-specific antibody (Suresh et al., Methods Enzymol. 121:210-228 (1986)), variable domains with the desired antibody-antigen combining sites are fused to Ig constant domain sequences. The fusion is preferably with an Ig heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. Preferably, the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding is in at least one of the fusions. DNAs encoding the Ig heavy-chain fusions and, if desired, the Ig light chain, are inserted into separate expression vectors and are co-transfected into a suitable host organism.

Fab fragments may be directly recovered from *E. coli* and chemically coupled to form bi-specific Abs. For example, fully humanized bi-specific F(ab') Abs can be produced (Shalaby et al., J Exp Med. 175:217-225 (1992)). Each Fab fragment is separately secreted from *E. coli* and directly coupled chemically in vitro, forming the bi-specific antibody.

Various techniques for making and isolating bi-specific antibody fragments directly from recombinant cell culture have also been described. For example, leucine zipper motifs can be exploited (Kostelny et al., Immunol. 148:1547-1553 (1992)). Peptides from the Fos and Jun proteins are linked to the Fab portions of two different Abs by gene fusion. The antibody homodimers are reduced at the hinge region to form monomers and then

reoxidized to form antibody heterodimers. This method can also produce antibody homodimers.

The "diabody" technology (Holliger et al., Proc Natl Acad Sci USA. 90:6444-6448 (1993)) provides an alternative method to generate bi-specific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker that is too short to allow pairing between the two domains on the same chain. The VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, forming two antigen-binding sites. Another strategy for making bi-specific antibody fragments is the use of single-chain Fv (sFv) dimers (Gruber et al., Immunol. 152:5368-5374 (1994)). Abs with more than two valencies are also contemplated, such as tri-specific Abs (Tutt et al., J Immunol. 147:60-69 (1991)).

Polyclonal Abs can be raised in a mammalian host, for example, by one or more injections of an immunogen and, if desired, an adjuvant. Typically, the immunogen and/or adjuvant are injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunogen may include AMIGO or an AMIGO fusion protein.

Examples of adjuvants include Freund's complete and monophosphoryl Lipid A synthetic-trehalose dicorynomycolate (MPL-TDM). To improve the immune response, an immunogen may be conjugated to a protein that is immunogenic in the AMIGO host, such as keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Protocols for antibody production are described by (Harlow et al, supra). Alternatively, pAbs may be made in chickens, producing IgY molecules (Schade et al, The production of avian (egg yolg) antibodies: IgY. The report and recommendations of ECVAM workshop. Alternatives to Laboratory Animals NAILA). 24:925-934 (1996)).

TREATMENT

The AMIGO protein, AMIGO gene, and AMIGO nucleic acids are believed to find ex vivo or in vivo therapeutic use for administration to a mammal, particularly humans, in the treatment of diseases or disorders, related to AMIGO activity or benefited by AMIGO-responsiveness. Particularly preferred are neurologic disorders, preferably central nervous

system disorders, Parkinson's disease, Alzheimer's disease, neuronal trauma or brain tumor.

The patient is administered an effective amount of AMIGO protein, biologically active peptide fragment, or variant of the invention or nucleic acids encoding said peptides. Therapeutic methods comprising administering AMIGO, AMIGO agonists, AMIGO antagonists or anti-AMIGO antibodies are within the scope of the present invention. The present invention also provides for pharmaceutical compositions comprising AMIGO protein, peptide fragment, or derivative in a suitable pharmacological carrier. The AMIGO protein, peptide fragment, or variant may be administered systemically or locally.

A disease or medical disorder is considered to be nerve damage if the survival or function of nerve cells and/or their axonal processes is compromised. Such nerve damage occurs as the result conditions including (a) Physical injury, which causes the degeneration of the axonal processes and/or nerve cell bodies near the site of the injury; (b) Ischemia, as a stroke; (c) Exposure to neurotoxins, such as the cancer and AIDS chemotherapeutic agents such as cisplatin and dideoxycytidine (ddC), respectively; (d) Chronic metabolic diseases, such as diabetes or renal dysfunction; and (e) Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Amyotrophic Lateral Sclerosis (ALS), which cause the degeneration of specific neuronal populations. Conditions involving nerve damage include Parkinson's disease, Alzheimer's disease, Amyotrophic Lateral Sclerosis, stroke, diabetic polyneuropathy, toxic neuropathy, glial scar, and physical damage to the nervous system such as that caused by physical injury of the brain and spinal cord or crush or cut injuries to the arm and hand or other parts of the body, including temporary or permanent cessation of blood flow to parts of the nervous system, as in stroke.

The invention features a method for treating a mammal who has suffered an injury to the central nervous system, such as stroke or a traumatic injury. The method involves administering an AMIGO protein, peptide fragment, or variant of the invention to the affected mammal at least six hours after onset of the injury; for example twelve, twenty-four, forty-eight hours, or even longer following injury. No practical end point the therapeutic window in which the invention can be practiced has yet been established. The invention can be used to treat one or more adverse consequences of central nervous system injury that arise from a variety of conditions. Thrombus, embolus, and systemic

hypotension are among the most common causes of stroke. Other injuries may be caused by hypertension, hypertensive cerebral vascular disease, rupture of an aneurysm, an angioma, blood dyscrasia, cardiac failure, cardiac arrest, cardiogenic shock, kidney failure, septic shock, head trauma, spinal cord trauma, seizure, bleeding from a tumor, or other loss of blood volume or pressure. These injuries lead to disruption of physiologic function, subsequent death of neurons, and necrosis (infarction) of the affected areas. The term "stroke" connotes the resulting sudden and dramatic neurologic deficits associated with any of the foregoing injuries.

The terms "ischemia" or "ischemic episode," as used herein, mean any circumstance that results in a deficient supply of blood to a tissue. Thus, a central nervous system ischemic episode results from an insufficiency or interruption in the blood supply to any locus of the brain such as, but not limited to, a locus of the cerebrum, cerebellum or brain stem. The spinal cord, which is also a part of the central nervous system, is equally susceptible to ischemia resulting from diminished blood flow. An ischemic episode may be caused by a constriction or obstruction of a blood vessel, as occurs in the case of a thrombus or embolus. Alternatively, the ischemic episode may result from any form of compromised cardiac function, including cardiac arrest, as described above. Where the deficiency is sufficiently severe and prolonged, it can lead to disruption of physiologic function, subsequent death of neurons, and necrosis (infarction) of the affected areas. The extent and type of neurologic abnormality resulting from the injury depend on the location and size of the infarct or the focus of ischemia. Where the ischemia is associated with a stroke, it can be either global or focal in extent.

It is expected that the invention will also be useful for treating traumatic injuries to the central nervous system that are caused by mechanical forces, such as a blow to the head. Trauma can involve a tissue insult selected from abrasion, incision, contusion, puncture, compression, etc., such as can arise from traumatic contact of a foreign object with any locus of or appurtenant to the mammalian head, neck or vertebral column. Other forms of traumatic injury can arise from constriction or compression of mammalian CNS tissue by an inappropriate accumulation of fluid (e.g., a blockade or dysfunction of normal cerebrospinal fluid or vitreous humor fluid production, turnover or volume regulation, or a subdural or intracranial hematoma or edema). Similarly, traumatic constriction or

compression can arise from the presence of a mass of abnormal tissue, such as a metastatic or primary tumor.

It is expected that the invention will also be useful for treating tumors or metastatic tumor cells, especially brain tumors. The most common brain tumors are gliomas, which begin in the glial tissue. Astrocytomas arise from small, star-shaped cells called astrocytes. In adults, astrocytomas most often arise in the cerebrum. A grade III astrocytoma is sometimes called anaplastic astrocytoma. A grade IV astrocytoma is usually called glioblastoma multiforme. Brain stem gliomas occur in the lowest, stemlike part of the brain. The brain stem controls many vital functions. Most brain stem gliomas are high-grade astrocytomas. Ependymomas usually develop in the lining of the ventricles. They may also occur in the spinal cord. Oligodendrogliomas arise in the cells that produce myelin, the fatty covering that protects nerves. These tumors usually arise in the cerebrum. They grow slowly and usually do not spread into surrounding brain tissue. Medulloblastomas develop from primitive nerve cells that normally do not remain in the body after birth. For this reason, medulloblastomas are sometimes called primitive neuroectodermal tumors (PNET). Most medulloblastomas arise in the cerebellum; however, they may occur in other areas as well. Meningiomas grow from the meninges. They are usually benign. Because these tumors grow very slowly, the brain may be able to adjust to their presence; meningiomas often grow quite large before they cause symptoms. They occur most often in women between 30 and 50 years of age. Schwannomas are benign tumors that begin in Schwann cells, which produce the myelin that protects the acoustic nerve. Acoustic neuromas are a type of schwannoma. Craniopharyngiomas develop in the region of the pituitary gland near the hypothalamus. They are usually benign; however, they are sometimes considered malignant because they can press on or damage the hypothalamus and affect vital functions. Germ cell tumors arise from primitive (developing) sex cells, or germ cells. The most frequent type of germ cell tumor in the brain is the germinoma. Pineal region tumors occur in or around the pineal gland. The tumor can be slow growing pineocytoma or fast growing (pineoblastoma). The pineal region is very difficult to reach, and these tumors often cannot be removed. Treatment for a brain tumor depends on a number of factors. Among these are the type, location, and size of the tumor, as well as the patient's age and general health. Normally brain tumors are treated with surgery, radiation therapy, and chemotherapy. Preferred tumours amenable for

AMIGO treatment express EGFR and are responsive to AMIGO mediated inhibition of EGFR phosphorylation.

The invention is suitable for the treatment of any primate, preferably a higher primate such as a human. In addition, however, the invention may be employed in the treatment of domesticated mammals which are maintained as human companions (e.g., dogs, cats, horses), which have significant commercial value (e.g., goats, pigs, sheep, cattle, sporting or draft animals), which have significant scientific value (e.g., captive or free specimens of endangered species, or inbred or engineered animal strains), or which otherwise have value. One of ordinary skill in the medical or veterinary arts is trained to recognize whether a mammal is afflicted with an ischemic or traumatic injury of the central nervous system. For example, routine testing and/or clinical or veterinary diagnostic evaluation will reveal whether the mammal has acquired an impairment or loss of central nervous system (e.g., neurologic) function. Clinical and non-clinical indications, as well as accumulated experience, relating to the presently disclosed and other methods of treatment, should appropriately inform the skilled practitioner in deciding whether a given individual is afflicted with an ischemic or traumatic injury of the central nervous system and whether any particular treatment is best suited to the subject's needs, including treatment according to the present invention.

In gene therapy applications, genes are introduced into cells in order to achieve in vivo synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes in vivo. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik et al., Proc. Natl. Acad. Sci. USA, 83:41434146 (1986)). The oligonucleotides can be modified to enhance their uptake, e.g., by substituting their negatively charged phosphodiester groups by uncharged groups.

Another technique for inhibiting the expression of a gene involves the use of RNA for induction of RNA interference (RNAi), using double stranded (dsRNA) (Fire *et al., Nature 391*: 806-811. 1998) or short-interfering RNA (siRNA) sequences (Yu *et al., Proc Natl Acad Sci U S A.* 99:6047-52. 2002). "RNAi" is the process by which dsRNA induces homology-dependent degradation of complimentary mRNA. In one embodiment, a synthetic antisense nucleic acid molecule is hybridized by complementary base pairing with a "sense" ribonucleic acid to form a double stranded RNA. The dsRNA antisense and sense nucleic acid molecules are provided that correspond to at least about 20, 25, 50, 100, 250 or 500 nucleotides or an entire AMIGO coding strand, or to only a portion thereof. In an alternative embodiment, the siRNAs are 30 nucleotides or less in length, and more preferably 21- to 23-nucleotides, with characteristic 2- to 3- nucleotide 3'-overhanging ends, which are generated by ribonuclease III cleavage from longer dsRNAs. (*See e.g.* Tuschl T. *Nat Biotechnol.* 20:446-48. 2002).

Intracellular transcription of small RNA molecules can be achieved by cloning the siRNA templates into RNA polymerase III (Pol III) transcription units, which normally encode the small nuclear RNA (snRNA) U6 or the human RNAse P RNA H1. Two approaches can be used to express siRNAs: in one embodiment, sense and antisense strands constituting the siRNA duplex are transcribed using constructs with individual promoters (Lee, *et al. Nat. Biotechnol.* 20, 500-505. 2002); in an alternative embodiment, siRNAs are expressed as stem-loop hairpin RNA structures that give rise to siRNAs after intracellular processing (Brummelkamp *et al. Science* 296:550-553. 2002) (herein incorporated by reference).

The dsRNA/siRNA is most commonly administered by annealing sense and antisense RNA strands *in vitro* before delivery to the organism. In an alternate embodiment, RNAi may be carried out by administering sense and antisense nucleic acids of the invention in the same solution without annealing prior to administration, and may even be performed by administering the nucleic acids in separate vehicles within a very close timeframe. Nucleic acid molecules encoding fragments and variants of an AMIGO or antisense nucleic acids complementary to an AMIGO nucleic acid sequence are additionally provided.

There are a variety of techniques available for introducing nucleic acids into viable cells.

The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, ex vivo, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, (Nicolau and Sene, *Biochim. Biophys. Acta*, 721:185-190 (1982); Fraley, *et al.*, *Proc. Natl. Acad. Sci. USA*, 76:3348-3352 (1979); Felgner, *Sci. Am.*, 276(6):102-6 (1997); Felgner, *Hum. Gene Ther.*, 7(15):1791-3, (1996)), electroporation (Tur-Kaspa, *et al.*, *Mol. Cell Biol.*, 6:716-718, (1986); Potter, *et al.*, *Proc. Nat. Acad. Sci. USA*, 81:7161-7165, (1984)), direct microinjection (Harland and Weintraub, *J. Cell Biol.*, 101:1094-1099 (1985)), cell fusion, DEAE-dextran (Gopal, *Mol. Cell Biol.*, 5:1188-1190 (1985), the calcium phosphate precipitation method (Graham and Van Der Eb, *Virology*, 52:456-467 (1973); Chen and Okayama, *Mol. Cell Biol.*, 7:2745-2752, (1987); Rippe, *et al.*, *Mol. Cell Biol.*, 10:689-695 (1990), cell sonication (Fechheimer, *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:8463-8467 (1987)), gene bombardment using high velocity microprojectiles (Yang, *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:9568-9572 (1990). The currently preferred in vivo gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau et al., Trends in Biotechnology, 11:205-210 (1993)). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g. capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu et al., J. Biol. Chem., 262:4429-4432 (1987); and Wagner et al., Proc. Natl. Acad. Sci. USA, 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson et al., Science, 256:808-813 (1992).

Any suitable vector may be used to introduce a transgene of interest into an animal. Exemplary vectors that have been described in the literature include replication-deficient retroviral vectors, including but not limited to lentivirus vectors [Kim et al., J. Virol., 72(1): 811-816 (1998); Kingsman & Johnson, Scrip Magazine, October, 1998, pp. 43-46.]; adenoviral (*see*, for example, U.S. Patent No. 5,824,544; U.S. Patent No. 5,707,618; U.S.

Patent No. 5,792,453; U.S. Patent No. 5,693,509; U.S. Patent No. 5,670,488; U.S. Patent No. 5,585,362; Quantin et al., Proc. Natl. Acad. Sci. USA, 89: 2581-2584 (1992); Stratford-Perricadet et al., J. Clin. Invest., 90: 626-630 (1992); and Rosenfeld et al., Cell, 68: 143-155 (1992)), retroviral (*see*, for example, U.S. Patent No. 5,888,502; U.S. Patent No. 5,830,725; U.S. Patent No. 5,770,414; U.S. Patent No. 5,686,278; U.S. Patent No. 4,861,719), adeno-associated viral (*see*, for example, U.S. Patent No. 5,474,935; U.S. Patent No. 5,139,941; U.S. Patent No. 5,622,856; U.S. Patent No. 5,658,776; U.S. Patent No. 5,773,289; U.S. Patent No. 5,789,390; U.S. Patent No. 5,834,441; U.S. Patent No. 5,863,541; U.S. Patent No. 5,851,521; U.S. Patent No. 5,252,479; Gnatenko et al., J. Investig. Med., 45: 87-98 (1997), an adenoviral-adenoassociated viral hybrid (*see*, for example, U.S. Patent No. 5,856,152) or a vaccinia viral or a herpesviral (*see*, for example, U.S. Patent No. 5,879,934; U.S. Patent No. 5,849,571; U.S. Patent No. 5,830,727; U.S. Patent No. 5,661,033; U.S. Patent No. 5,328,688); Lipofectin-mediated gene transfer (BRL); liposomal vectors [See, e.g., U.S. Patent No. 5,631,237 (Liposomes comprising Sendai virus proteins)] ; and combinations thereof. All of the foregoing documents are incorporated herein by reference in the entirety. Replication-deficient adenoviral vectors, adeno-associated viral vectors and lentiviruses constitute preferred embodiments.

In embodiments employing a viral vector, preferred polynucleotides include a suitable promoter and polyadenylation sequence to promote expression in the target tissue of interest. For many applications of the present invention, suitable promoters/enhancers for mammalian cell expression include, e.g., cytomegalovirus promoter/enhancer [Lehner et al., J. Clin. Microbiol., 29:2494-2502 (1991); Boshart et al., Cell, 41:521-530 (1985)]; Rous sarcoma virus promoter [Davis et al., Hum. Gene Ther., 4:151 (1993)]; simian virus 40 promoter, long terminal repeat (LTR) of retroviruses, keratin 14 promoter, and α myosin heavy chain promoter.

In a particular embodiment of the invention, the expression construct (or the peptides discussed above) may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures

and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, "In Liver Diseases, Targeted Diagnosis And Therapy Using Specific Receptors And Ligands," Wu, G., Wu, C., ed., New York: Marcel Dekker, pp. 87-104 (1991)). The addition of DNA to cationic liposomes causes a topological transition from liposomes to optically birefringent liquid-crystalline condensed globules (Radler, *et al.*, *Science*, 275(5301):810-4, (1997)). These DNA-lipid complexes are potential non-viral vectors for use in gene therapy and delivery.

Also contemplated in the present invention are various commercial approaches involving "lipofection" technology. In certain embodiments of the invention, the liposome may be complexed with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (Kaneda, *et al.*, *Science*, 243:375-378 (1989)). In other embodiments, the liposome may be complexed or employed in conjunction with nuclear nonhistone chromosomal proteins (HMG-1) (Kato, *et al.*, *J. Biol. Chem.*, 266:3361-3364 (1991)). In yet further embodiments, the liposome may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression constructs have been successfully employed in transfer and expression of nucleic acid *in vitro* and *in vivo*, then they are applicable for the present invention.

Other vector delivery systems that can be employed to deliver a nucleic acid encoding a therapeutic gene into cells include receptor-mediated delivery vehicles. These take advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost all eukaryotic cells. Because of the cell type-specific distribution of various receptors, the delivery can be highly specific (Wu and Wu (1993), *supra*).

In another embodiment of the invention, the expression construct may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above that physically or chemically permeabilize the cell membrane. This is applicable particularly for transfer *in vitro*, however, it may be applied for *in vivo* use as well. Dubensky, *et al.*, *Proc. Nat. Acad. Sci. USA*, 81:7529-7533 (1984) successfully injected polyomavirus DNA in the form of $CaPO_4$ precipitates into liver and

spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Neshif, *Proc. Nat. Acad. Sci. USA*, 83:9551-9555 (1986) also demonstrated that direct intraperitoneal injection of $CaPO_4$ precipitated plasmids results in expression of the transfected genes.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein, *et al.*, *Nature*, 327:70-73 (1987)). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang, *et al.*, *Proc. Natl. Acad. Sci USA*, 87:9568-9572 (1990)). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

Those of skill in the art are aware of how to apply gene delivery to *in vivo* and *ex vivo* situations. For viral vectors, one generally will prepare a viral vector stock. Depending on the type of virus and the titer attainable, one will deliver 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}, 1×10^{11} or 1×10^{12} infectious particles to the patient. Similar figures may be extrapolated for liposomal or other non-viral formulations by comparing relative uptake efficiencies. Formulation as a pharmaceutically acceptable composition is discussed below.

Various routes are contemplated for various cell types. For practically any cell, tissue or organ type, systemic delivery is contemplated. In other embodiments, a variety of direct, local and regional approaches may be taken. For example, the cell, tissue or organ may be directly injected with the expression vector or protein.

In a different embodiment, *ex vivo* gene therapy is contemplated. In an *ex vivo* embodiment, cells from the patient are removed and maintained outside the body for at

least some period of time. During this period, a therapy is delivered, after which the cells are reintroduced into the patient.

The invention also provides antagonists of AMIGO activation (e.g., AMIGO antisense nucleic acid, RNAi, neutralizing antibodies). Administration of AMIGO antagonist to a mammal having increased or excessive levels of endogenous AMIGO activation is contemplated, preferably in the situation where such increased levels of AMIGO lead to a pathological disorder.

PHARMACEUTICAL AND THERAPEUTICAL COMPOSITIONS AND FORMULATIONS

The AMIGO nucleic acid molecules, AMIGO polypeptides, AMIGO agonists, AMIGO antagonists and anti-AMIGO Abs (active compounds) of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions.

Such compositions of AMIGO are prepared for storage by mixing AMIGO nucleic acid molecule, protein, or antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A., Ed., (1980)), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

The AMIGO nucleic acid molecule, protein, agonist, antagonist or antibodies may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, supra.

The route of AMIGO nucleic acid molecule, protein, or antibody administration is in accord with known methods, e.g., those routes set forth above for specific indications, as well as the general routes of injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intrathecal, intracranial, intraspinal, intraventricular, intraarterial, or intralesional means, or sustained release systems as noted below. AMIGO nucleic acid molecule, protein, or antibody is administered continuously by infusion or by bolus injection. Generally, where the disorder permits, one should formulate and dose the AMIGO nucleic acid molecule, protein, or antibody for site-specific delivery. Administration can be continuous or periodic. Administration can be accomplished by a constant- or programmable-flow implantable pump or by periodic injections. The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (Nabel and Nabel, US Patent No. 5, 328, 470, 1994), or by stereotactic injection (Chen et al., Proc. Natl. Acad. Sci. USA 91:3054-3057 (1994)). The pharmaceutical preparation of a gene therapy vector can include an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded.

Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels as described by Langer et al., J. Biomed. Mater. Res.,

15:167-277 (1981) and Langer, Chem. Tech., 12:98-105 (1982) or polyvinylalcohol, polylactides (U.S. Pat. No. 3,773,919, EP 58,481), or non-degradable ethylene-vinyl acetate (Langer et al., supra).

Sustained-release AMIGO compositions also include liposomally entrapped AMIGO nucleic acid molecule, protein, agonist, antagonist or antibodies. Liposomes containing AMIGO nucleic acid molecule, protein, or antibodies are prepared by methods known per se: Epstein et al., Proc. Natl. Acad. Sci. USA, 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77:40304034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol % cholesterol, the selected proportion being adjusted for the optimal AMIGO nucleic acid molecule, protein, or antibody therapy.

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37 °C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Semipermeable, implantable membrane devices are useful as means for delivering drugs in certain circumstances. For example, cells that secrete soluble AMIGO or express AMIGO on their cell surface, chimeras or antibodies can be encapsulated, and such devices can be implanted into a patient. For example, into the brain of patients suffering from Parkinson's Disease, neuronal trauma or glial scar. See, U.S. Pat. No. 4,892,538 of Aebischer et al.; U.S. Pat. No. 5,011,472 of Aebischer et al.; U.S. Pat. No. 5,106,627 of Aebischer et al.; PCT Application WO 91/10425; PCT Application WO 91/10470; Winn et al., Exper. Neurology, 113:322-329 (1991); Aebischer et al., Exper Neurology, 111:269-275 (1991); and Tresco et al., ASAIO, 38:17-23 (1992).

Accordingly, also included is a method for preventing or treating damage to a nerve or damage to other AMIGO-responsive cells, which comprises implanting cells that secrete AMIGO or express AMIGO on their cell surface, its agonists or antagonists as may be required for the particular condition, into the body of patients in need thereof. Finally, the present invention includes a device for preventing or treating nerve damage or damage to other cells as taught herein by implantation into a patient comprising a semipermeable membrane, and a cell that secretes AMIGO (or its agonists or antagonists as may be required for the particular condition) encapsulated within said membrane and said membrane being permeable to AMIGO (or its agonists or antagonists) and impermeable to factors from the patient detrimental to the cells. The patient's own cells, transformed to produce AMIGO ex vivo, could be implanted directly into the patient, optionally without such encapsulation. The methodology for the membrane encapsulation of living cells is familiar to those of ordinary skill in the art, and the preparation of the encapsulated cells and their implantation in patients may be accomplished without under experimentation.

The present invention includes, therefore, a method for preventing or treating nerve damage by implanting cells, into the body of a patient in need thereof, cells either selected for their natural ability to generate or engineered to secrete AMIGO or AMIGO antibody. Preferably, the expressed or secreted AMIGO or antibody being soluble, human mature AMIGO when the patient is human. The implants are preferably non-immunogenic and/or prevent immunogenic implanted cells from being recognized by the immune system. For CNS delivery, a preferred location for the implant is the cerebral spinal fluid of the spinal cord.

An effective amount of AMIGO nucleic acid molecule, protein, agonist, antagonist or antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titre the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the AMIGO protein or antibody until a dosage is reached that achieves the desired effect. A typical daily dosage for systemic treatment might range from about 1 microgram/kg to up to 10 mg/kg or more, depending on the factors mentioned above. As an alternative general proposition, the AMIGO nucleic acid molecule, protein, or antibody is formulated and

delivered to the target site or tissue at a dosage capable of establishing in the tissue an AMIGO level that is efficacious but not unduly toxic. This intra-tissue concentration should be maintained if possible by continuous infusion, sustained release, topical application, AMIGO-expressing cell implant, or injection at empirically determined frequencies. The progress of this therapy is easily monitored by conventional assays.

As will be appreciated by one of ordinary skill in the art, the formulated compositions contain therapeutically-effective amounts of the AMIGO protein, peptide fragment, or variant of the invention or modulator of AMIGO receptors. That is, they contain an amount which provides appropriate concentrations of the agent to the affected nervous system tissue for a time sufficient to stimulate a detectable restoration of central nervous system function, up to and including a complete restoration thereof. As will be appreciated by those skilled in the art, these concentrations will vary depending upon a number of factors, including the biological efficacy of the selected agent, the chemical characteristics (e.g., hydrophobicity) of the specific agent, the formulation thereof, including a mixture with one or more excipients, the administration route, and the treatment envisioned, including whether the active ingredient will be administered directly into a tissue site, or whether it will be administered systemically. The preferred dosage to be administered also is likely to depend on such variables such as the condition of the diseased or damaged tissues, and the overall health status of the particular mammal. As a general matter, single, daily, biweekly or weekly dosages of 0.00001-1000 mg of an AMIGO protein, peptide fragment, or variant of the invention or agonists of AMIGO receptors are sufficient with 0.0001-100 mg being preferable, and 0.001 to 10 mg being even more preferable. Alternatively, a single, daily, biweekly or weekly dosage of 0.01-1000 .mu.g/kg body weight, more preferably 0.01-10 mg/kg body weight, may be advantageously employed. The present effective dose can be administered in a single dose or in a plurality (two or more) of installment doses, as desired or considered appropriate under the specific circumstances. A bolus injection or diffusable infusion formulation can be used. If desired to facilitate repeated or frequent infusions, implantation of a semi-permanent stent (e.g., intravenous, intraperitoneal, intracisternal or intracapsular) may be advisable. In Example below, intraspinal administration of AMIGO, AMIGO2 or AMIGO3 confer clearly detectable levels of restoration of lost or impaired central nervous system function.

USES OF AMIGO COMPOUNDS

The present invention employs AMIGO compounds for use in inhibiting the function of EGFR, ultimately modulating the phosphorylation of EGFR and thus modulating the signalling cascade initiated by EGFR. This is accomplished by providing AMIGO compounds which specifically bind and modulate EGFR phosphorylation. Such AMIGO compounds interfere with the normal role of EGFR function and causes a modulation of its cellular signaling. The functions of EGFR phosphorylation to be interfered include all vital functions such as, for example, ligand-receptor interaction, dimerization of EGFR in the cell membrane, phosphorylation of EGFR, modulation of EGFR initiated signalling cascades which may be engaged in by EGFR. The overall effect of such interference with AMIGO compounds is modulation of the phosphorylation of EGFR. In the context of this invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the phosphorylation of an EGFR. In the context of the present invention, inhibition is the preferred form of modulation of EGFR phosphorylation.

Some of the featured AMIGO compounds can be used to treat cell proliferative disorders characterized by inappropriate EGFR activity. "Inappropriate EGFR" activity refers to either: 1) EGF-receptor (EGFR) expression in cells which normally do not express EGFR; 2) EGF expression by cells which normally do not express EGF/TGF-.alpha.; 3) increased EGF-receptor (EGFR) expression leading to unwanted cell proliferation; 4) increased EGF/TGF-.alpha. expression leading to unwanted cell proliferation; and/or 5) mutations leading to constitutive activation of EGF-receptor (EGFR). The existence of inappropriate or abnormal EGF/TGF-.alpha. and EGFR levels or activities is determined by procedures well known in the art.

An increase in EGF/TGF-.alpha. activity or expression is characterized by an increase in one or more of the activities which can occur upon EGF ligand binding such as: (1) EGF-R dimerization; (2) auto-phosphorylation of EGFR, (3) phosphorylation of an EGFR substrate (e.g., PLC.gamma.), (4) activation of an adapter molecule, and/or (5) increased cell division. These activities can be measured using techniques described below and known in the art. For example auto-phosphorylation of EGFR can be measured using an anti-phosphotyrosine antibody, and increased cell division can be performed by measuring .sup.3 H-thymidine incorporation into DNA. Preferably, the increase in EGFR activity is

characterized by an increased amount of phosphorylated EGFR and/or DNA synthesis.

Unwanted cell proliferation can result from inappropriate EGFR activity occurring in different types of cells including cancer cells, cells surrounding a cancer cell, and endothelial cells. Examples of disorders characterized by inappropriate EGF activity include cancers such as glioma, head, neck, gastric, lung, breast, ovarian, colon, and prostate.

AMIGO compound. The term "AMIGO compound" is meant to refer to an AMIGO peptide, variants, biologically active fragments, antigenic fragment, anti-AMIGO antibodies or binding portion thereof and nucleic acids encoding said peptides which are capable of binding to or interacting in some way with EGFR or a ligand of the epidermal growth factor receptor. Binding or interaction of an AMIGO compound of the invention with the corresponding ligand results in the modulation, preferably prevention or inhibition, of the interaction between a ligand and its corresponding receptor. Because the ligand-receptor interaction is involved in the proliferation of EGFR-expressing tumour cells, the term "an AMIGO compound" is meant to include all compounds which modulate the interaction between the epidermal growth factor receptor and their corresponding ligands, more preferably adversely affect interaction between the epidermal growth factor receptor and their corresponding ligands leading to inhibition of phosphorylation of EGFR.

As used herein, the terms "inhibits phosphorylation" (e.g., referring to inhibition/blocking of phosphorylation of EGFR) encompass both partial and complete inhibition. The inhibition of EGFR phosphorylation preferably reduces or alters the normal level or type of cell signaling that occurs when EGFR ligand binds to EGFR without inhibition or blocking. Inhibition is also intended to include any measurable decrease in the binding affinity of EGFR ligand to EGFR when in contact with an AMIGO compound as compared to the ligand not in contact with an AMIGO compound, e.g., the blocking of EGFR ligands to EGFR by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%.

The AMIGO compounds of the present invention have a multitude of therapeutic and diagnostic uses. For example, therapeutic uses involve cancer therapy in a patient suspected of suffering from cancer or other related diseases. Specifically, AMIGO

compounds of the present invention can be used to treat patients that have tumour cells which produce the EGFR ligand and/or overexpress the EGFR proteins.

One type of treatment may involve the use of the AMIGO compounds coupled to a therapeutic agent. By administering an effective amount of AMIGO compounds coupled with the therapeutic agent to a patient, a tumour cells in the patient which express EGFR can be growth inhibited or killed, thereby providing a treatment for cancer.

In accordance with the method of cancer treatment of the invention, the conjugated AMIGO compound is capable of recognizing and binding to tumour cells due to the association of the tumour cells with the EGFR. Without being limited, the mechanism of binding to the cancer cell may involve the recognition of EGFR, ligand located on the cell surface or because of expression and/or secretion of the ligand.

Once the conjugated AMIGO compound is bound or in close association with the tumour cell by interacting with EGFR, the therapeutic agent is capable of inhibiting or killing that cell. In this manner, the therapy of the present invention is selective for a particular target, e.g., cancer cells which are associated with the EGFR.

Normal cells and other cells not associated with the EGFR (cells which do not express EGFR) may not, for the most part, be affected by therapy with AMIGO compounds.

Alternatively, the AMIGO compounds of the present invention may be used to prevent or inhibit inducement of tumour cell proliferation. For example, cancer cells which contain the EGFR are induced to proliferate in the presence of low concentrations of EGFR ligand. Preventing the EGFR ligand from interacting with its receptor may provide a means to treat a cancer patient.

According to the method of inhibiting or preventing cellular proliferation of the present invention, the AMIGO compound is capable of binding to the EGFR. Binding the EGFR in vivo forms an EGFR- AMIGO compound complex and thus may prevent or inhibit the ligand-receptor interaction either sterically or otherwise. Thus, the present invention provides a treatment to prevent or inhibit tumour cell proliferation in a patient by administering an effective amount of an AMIGO compound to such a patient.

It will be appreciated that a number of other therapeutic uses of the AMIGO compounds of the present invention may be devised. Such therapies may involve use of other known treatment techniques in combination with the AMIGO compounds of the invention. The present invention is not meant to be limited to the therapeutic treatment described and are thus only presented by way of illustration.

Furthermore, administration of an amount of the AMIGO compounds of the present invention sufficient to inhibit or kill a tumour cell may vary depending upon a number of factors including the type of malignant cell, body weight of the patient, the type of therapeutic agent used and the like. Those of skill in the art will appreciate that the amount necessary to inhibit or kill a particular malignant cell in vitro or in vivo can easily be determined with minimal routine experimentation. An effective amount of such AMIGO compounds may be administered parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally or orally. In addition, pharmaceutical preparations may be prepared which contain suitable excipients, auxiliaries, or compounds which facilitate processing or stability of the AMIGO compounds of the invention as pharmaceutical agents.

Diagnostic uses of the AMIGO compounds of the present invention (due to its modification of EGFR phosphorylation) may include, for example, detection of EGFR in a sample obtained from a patient. Such samples may be body tissue, body fluids (such as blood, urine, tear drops, saliva, serum, and cerebrospinal fluid), feces, cellular extracts and the like.

Assaying for the EGFR phosphorylation of the invention in a sample obtained from a patient may thus provide for a method for diagnosing cancer. That is, detection of EGFR in a sample obtained from a patient indicates the presence of EGFR expressing cells in a patient. Furthermore, since the AMIGO compound is specific for, EGFR, the phosphorylation assay may provide information concerning the biology of a patient's tumor. For example, cancer patients with a tumour cells that overexpress the EGFR are known to have poorer overall survival than cancer patients that do not show EGFR overexpression. Detection of EGFR phosphorylation may thus serve as a prognostic test, allowing the clinician to select a more effective therapy for treating the patient.

The AMIGO compound compositions of the invention can be initially tested for binding activity associated with therapeutic or diagnostic use in vitro. For example, compositions of the invention can be tested using the ELISA and flow cytometric assays described in the Examples below. Moreover, the activity of these molecules in triggering at least one effector-mediated effector cell activity, including phosphorylation of EGFR of cells expressing EGFR can be assayed.

The compositions of the invention have additional utility in therapy and diagnosis of EGFR-related diseases. For example, the AMIGO DNA can be used to elicit in vivo or in vitro one or more of the following biological activities: to inhibit EGF or TGF-.alpha. induced autophosphorylation in a cell expressing EGFR; to inhibit autocrine EGF or TGF-.alpha.-induced activation of a cell expressing EGFR; or to inhibit the growth of a cell expressing EGFR, e.g., at low dosages.

In a particular embodiment, the AMIGO compounds and derivatives/variants thereof are used in vivo to treat, prevent or diagnose a variety of EGFR-related diseases. Examples of EGFR-related diseases include a variety of cancers, such as glioma, glioblastoma, bladder, breast, uterine/cervical, colon, pancreatic, colorectal, kidney, stomach, ovarian, prostate, renal cell, squamous cell, lung (non-small cell), esophageal, and head and neck cancer.

Methods of administering the compositions of the invention are known in the art. Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. The AMIGO compounds can be coupled to radionuclides, such as 131I, 90Y, 105Rh, indium-111, etc., as described in Goldenberg, D. M. et al. (1981) Cancer Res. 41: 4354-4360, and in EP 0365 997. In another aspect the invention relates to an immunoconjugate comprising an AMIGO antibody or binding portion thereof or AMIGO peptide or fragment according to the invention linked to a radioisotope, cytotoxic agent (e.g., calicheamicin and duocarmycin), a cytostatic agent, or a chemotherapeutic drug. The compositions of the invention can also be coupled to anti-infectious agents.

In another embodiment, the AMIGO compounds can be co-administered with a therapeutic agent, e.g., a chemotherapeutic agent, an immunosuppressive agent, or can be co-administered with other known therapies, such as physical therapies, e.g., radiation therapy, hyperthermia, or transplantation (e.g., bone marrow transplantation). Such

therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/m.sup.2 dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/m.sup.2 dose once every 21 days.

Pharmaceutical compositions of the present invention can include one or more further chemotherapeutic agents selected from the group consisting of nitrogen mustards (e.g., cyclophosphamide and ifosfamide), aziridines (e.g., thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine and streptozocin), platinum complexes (e.g., carboplatin and cisplatin), non-classical alkylating agents (e.g., dacarbazine and temozolamide), folate analogs (e.g., methotrexate), purine analogs (e.g., fludarabine and mercaptopurine), adenosine analogs (e.g., cladribine and pentostatin), pyrimidine analogs (e.g., fluorouracil (alone or in combination with leucovorin) and gemcitabine), substituted ureas (e.g., hydroxyurea), antitumor antibiotics (e.g., bleomycin and doxorubicin), epipodophyllotoxins (e.g., etoposide and teniposide), microtubule agents (e.g., docetaxel and paclitaxel), camptothecin analogs (e.g., irinotecan and topotecan), enzymes (e.g., asparaginase), cytokines (e.g., interleukin-2 and interferon-.alpha.), monoclonal antibodies (e.g., trastuzumab and bevacizumab), recombinant toxins and immunotoxins (e.g., recombinant cholera toxin-B and TP-38), cancer gene therapies, physical therapies (e.g., hyperthermia, radiation therapy, and surgery) and cancer vaccines (e.g., vaccine against telomerase).

Co-administration of the AMIGO compounds of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

In another embodiment, the subject can be additionally treated with a lymphokine preparation. Cancer cells which do not highly express EGFR can be induced to do so using lymphokine preparations. Lymphokine preparations can cause a more homogeneous expression of EGFRs among cells of a tumor which can lead to a more effective therapy.

Lymphokine preparations suitable for administration include interferon-gamma, tumor necrosis factor, and combinations thereof. These can be administered intravenously. Suitable dosages of lymphokine are 10,000 to 1,000,000 units/patient.

In one embodiment, the invention provides methods for detecting the presence of EGFR phosphorylation in a sample, or measuring the amount of EGFR phosphorylation, comprising contacting the sample, and a control sample, with an AMIGO compound, which specifically binds to EGFR, under conditions that allow for formation of a complex between the AMIGO compound and EGFR. The formation of a complex is then detected, i.e. modulation, preferably inhibition of phosphorylation, wherein a difference in EGFR phosphorylationbetween the sample compared to the control sample is indicative the presence of EGFR in the sample.

SCREENING

The present invention also encompasses agent which modulate AMIGO expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per

kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

The subject methods include screens for agents which modulate homophilic or heterophilic AMIGO interactions and methods for modulating these interactions. AMIGO activation is found to regulate a wide variety of cell functions, including cell-cell interactions, cell mobility, neurite growth and fasciculation. AMIGO polypeptides are disclosed as specific modulators of function of EGFR polypeptides. Accordingly, the invention provides methods for modulating targeted cell function comprising the step of modulating AMIGO activation by contacting the cell with a modulator of a AMIGO:AMIGO or AMIGO:AMIGO ligand interaction. The invention also provides methods for modulating targeted cell function comprising the step of modulating EGFR activation by contacting the cell with a modulator of a AMIGO:EGFR interaction.

In another aspect, the invention provides methods of screening for agents which modulate AMIGO:AMIGO, AMIGO:EGFR or AMIGO:AMIGO ligand interactions. These methods generally involve forming a mixture of an AMIGO-expressing cell, an AMIGO, EGFR or AMIGO ligand polypeptide and a candidate agent, and determining the effect of the agent on the amount of AMIGO expressed by the cell. The methods are amenable to automated, cost-effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and human trials; for example, the reagents may be derivatized and rescreened in vitro and in vivo assays to

optimize activity and minimize toxicity for pharmaceutical development. More specifically, neuronal cell based neural outgrowth assays, fasciculation and aggregation assays are described in detail in the experimental section below.

The invention further provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to AMIGO proteins, have a stimulatory or inhibitory effect on, for example, AMIGO expression or AMIGO activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of an AMIGO substrate. Compounds thus identified can be used to modulate the activity of AMIGOs in a therapeutic protocol, to elaborate the biological function of the AMIGO, or to identify compounds that disrupt normal AMIGO interactions. The preferred AMIGOs used in this embodiment are the AMIGO, AMIGO2 and AMIGO3 of the present invention.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of an AMIGO protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of an AMIGO protein or polypeptide or biologically active portion thereof.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries [libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive] (see, e.g., Zuckermann, R. N. et al. J. Med. Chem. 1994, 37: 2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the `one-bead one-compound` library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K. S. (1997) Anticancer Drug Des. 12:145).

In one embodiment, an assay is a cell-based assay in which a cell which expresses an AMIGO protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate AMIGO activity is determined. Determining the ability of the test compound to modulate AMIGO activity can be accomplished by monitoring, for example, cell attachment or adhesion, cell growth, neurite outgrowth, fasciculation and cell chemotaxis. The cell, for example, can be of mammalian origin, e.g., a neuronal cell. In preferred embodiment, AMIGO is expressed in neuronal cells and the ability of the test compound to modulate AMIGO activity is accomplished by monitoring neurite outgrowth or alternatively, by monitoring axonal fasciculation. In another prererred embodiment AMIGO and EGFR are co-expressed, e.g. in tumour cells of neuronal or non-neuronal origin, and the amount of phosphorylation of EGFR in monitored.

Determining the ability of the AMIGO protein or a biologically active fragment thereof, to bind to or interact with an AMIGO target molecule (comprising for example AMIGO, EGFR or AMIGO ligand) can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the AMIGO protein to bind to or interact with an AMIGO target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (i.e., intracellular calcium or IP3), detecting catalytic/enzymatic activity of the target molecule upon an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a target-regulated cellular response (i.e., cell attachment, adhesion, growth or migration).

In yet another embodiment, an assay of the present invention is a cell-free assay in which an AMIGO protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the AMIGO protein or biologically active portion thereof is determined. Preferred biologically active portions of the AMIGO proteins to be used in assays of the present invention include fragments which participate in interactions with AMIGO, EGFR or AMIGO ligand protein. Preferably, these fragments comprise extracellular parts of the AMIGO or EGFR proteins.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated AMIGO proteins or biologically active portions thereof. In the case of cell-free assays in which a membrane-bound form of an AMIGO protein is used it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton.RTM. X-100, Triton.RTM. X-114, Thesit.RTM., Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

The principle of the assays used to identify compounds that bind to the AMIGO protein involves preparing a reaction mixture of the AMIGO protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay would involve anchoring AMIGO protein or the test substance onto a solid phase and detecting AMIGO protein/test compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, AMIGO protein can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein and which are well-known to one skilled in the art.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either AMIGO, EGFR or AMIGO ligand to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to an AMIGO protein, or interaction of an AMIGO protein with AMIGO, EGFR or AMIGO ligand in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/AMIGO fusion proteins or glutathione-S-transfera- se/target

fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed AMIGO, EGFR or AMIGO ligand protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of AMIGO binding or activity determined using standard techniques.

In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously nonimmobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with AMIGO protein, EGFR or AMIGO ligand but which do not interfere with binding of the AMIGO protein to AMIGO, EGFR or AMIGO ligand. Such antibodies can be derivatized to the wells of the plate, and AMIGO, EGFR, or AMIGO ligand trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the AMIGO, EGFR or AMIGO ligand, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the AMIGO protein, EGFR or AMIGO ligand.

Alternatively, in another embodiment, an assay can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a

number of standard techniques, including but not limited to: differential centrifugation, chromatography, electrophoresis and immunoprecipitation. In differential centrifugation, complexes of molecules may be separated from uncomplexed molecules through a series of centrifugal steps, due to the different sedimentation equilibria of complexes based on their different sizes and densities (see, for example, Rivas, G., and Minton, A. P., Trends Biochem Sci 1993 Aug;18(8):284-7). Standard chromatographic techniques may also be utilized to separate complexed molecules from uncomplexed ones. For example, gel filtration chromatography separates molecules based on size, and through the utilization of an appropriate gel filtration resin in a column format, for example, the relatively larger complex may be separated from the relatively smaller uncomplexed components. Similarly, the relatively different charge properties of the complex as compared to the uncomplexed molecules may be exploited to differentially separate the complex from the remaining individual reactants, for example through the use of ion-exchange chromatography resins. Such resins and chromatographic techniques are well known to one skilled in the art (see, e.g., Heegaard, N. H., J Mol Recognit 1998 Winter;11(1-6):141-8; Hage, D. S., and Tweed, S. A. J Chromatogr B Biomed Sci Appl 1997 Oct 10;699(1-2):499-525). Gel electrophoresis may also be employed to separate complexed molecules from unbound species (see, e.g., Ausubel, F. et al., eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York.). In this technique, protein or nucleic acid complexes are separated based on size or charge, for example. In order to maintain the binding interaction during the electrophoretic process, nondenaturing gels in the absence of reducing agent are typically preferred, but conditions appropriate to the particular interactants will be well known to one skilled in the art. Immunoprecipitation is another common technique utilized for the isolation of a protein-protein complex from solution (see, for example, Ausubel, F. et al., eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York). In this technique, all proteins binding to an antibody specific to one of the binding molecules are precipitated from solution by conjugating the antibody to a polymer bead that may be readily collected by centrifugation. The bound proteins are released from the beads (through a specific proteolysis event or other technique well known in the art which will not disturb the protein-protein interaction in the complex), and a second immunoprecipitation step is performed, this time utilizing antibodies specific for a different interacting protein. In this manner, only the complex should remain attached to the beads. The captured complex may be visualized using gel electrophoresis. The presence of a molecular complex (which may be identified by any of these techniques)

indicates that a specific binding event has occurred, and that the introduced compound specifically binds to the target protein. Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the AMIGO protein or biologically active portion thereof with a known compound which binds AMIGO to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an AMIGO protein, wherein determining the ability of the test compound to interact with an AMIGO protein comprises determining the ability of the test compound to preferentially bind to AMIGO or biologically active portion thereof as compared to the known compound. In further preferred embodiment, AMIGO protein or biologically active portion thereof is contacted with AMIGO protein and the ability of the test compound to interact with AMIGO is compared to known AMIGO:AMIGO interaction. In a still further embodiment, AMIGO protein or biologically active portion thereof is contacted with EGFR protein and the ability of the test compound to interact with AMIGO is compared to known AMIGO:EGFR interaction.

In yet another embodiment, the cell-free assay involves contacting an AMIGO protein or biologically active portion thereof with a known compound which binds the AMIGO protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the AMIGO protein, wherein determining the ability of the test compound to interact with the AMIGO protein comprises determining the ability of the AMIGO protein to preferentially bind to or modulate the activity of an AMIGO, EGFR or AMIGO ligand.

The AMIGO proteins of the invention can, in vivo, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the AMIGOs. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred proteins for use in this embodiment are the AMIGO proteins herein identified. Towards this purpose, in an alternative embodiment, the invention provides methods for determining the ability of the

test compound to modulate the activity of an AMIGO protein through modulation of the activity of a downstream effector of an AMIGO, EGFR or AMIGO ligand. For example, the activity of the effector molecule on an AMIGO, EGFR or AMIGO ligand can be determined, or the binding of the effector to AMIGO, EGFR or AMIGO ligand can be determined as previously described.

The basic principle of the assay systems used to identify compounds that interfere with the interaction between the AMIGO and its cellular or extracellular binding partner or partners involves preparing a reaction mixture containing the AMIGO, and the binding partner under conditions and for a time sufficient to allow the two products to interact and bind, thus forming a complex. In order to test an agent for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the AMIGO and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the AMIGO and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the AMIGO and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and AMIGO can also be compared to complex formation within reaction mixtures containing the test compound and mutant AMIGO. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal AMIGOs.

The assay for compounds that interfere with the interaction of the AMIGOs and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the AMIGO or the binding partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the AMIGOs and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance; i.e., by adding the test

substance to the reaction mixture prior to or simultaneously with the AMIGO and interactive cellular or extracellular binding partner. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the AMIGO or the interactive cellular or extracellular binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtitre plates are conveniently utilized. The anchored species can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished simply by coating the solid surface with a solution of the AMIGO or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of

addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the AMIGO and the interactive cellular or extracellular binding partner product is prepared in that either the AMIGOs or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Pat. No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt AMIGO-cellular or extracellular binding partner interaction can be identified.

Assays for the Detection of the Ability of a Test Compound to Modulate Expression of AMIGO

In another embodiment, modulators of AMIGO expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of AMIGO mRNA or protein in the cell is determined. The level of expression of AMIGO mRNA or protein in the presence of the candidate compound is compared to the level of expression of AMIGO mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of AMIGO expression based on this comparison. For example, when expression of AMIGO mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of AMIGO mRNA or protein expression. Alternatively, when expression of AMIGO mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of AMIGO mRNA or protein expression. The level of AMIGO mRNA or protein expression in the cells can be determined by methods described herein for detecting AMIGO mRNA or protein.

Combination Assays

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of an AMIGO protein can be confirmed in vivo, e.g., in an animal such as an animal model for CNS disorders, or for cellular transformation and/or neuronal regeneration.

This invention farther pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., an AMIGO modulating agent, an antisense AMIGO nucleic acid molecule, an AMIGO-specific antibody, or an AMIGO-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

The choice of assay format will be based primarily on the nature and type of sensitivity/resistance protein being assayed. A skilled artisan can readily adapt protein activity assays for use in the present invention with the genes identified herein.

DIAGNOSTICS

The invention also features diagnostic or prognostic kits for use in detecting the presence of AMIGO or allelic variant thereof in a biological sample. The kit provides means for the diagnostics of AMIGO dependent conditions as described hereinabove or for assessing the predisposition of an individual to conditions mediated by variation or dysfunction of AMIGO. The kit can comprise a labeled compound capable of detecting AMIGO polypeptide or nucleic acid (e.g. mRNA) in a biological sample. The kit can also comprise nucleic acid primers or probes capable of hybridising specifically to at least of portion of an AMIGO gene or allelic variant thereof. The kit can be packaged in a suitable container and preferably it contains instructions for using the kit.

PURIFICATION OF AMIGO BINDING MOLECULES

In yet another aspect of the invention, the AMIGO or AMIGO analog may be used for affinity purification of molecules (receptors) that bind to the AMIGO. AMIGO is a preferred ligand for purification. Briefly, this technique involves: (a) contacting a source of AMIGO receptor with an immobilized AMIGO under conditions whereby the AMIGO receptor to be purified is selectively adsorbed onto the immobilized AMIGO; (b) washing the immobilized AMIGO and its support to remove non-adsorbed material; and (c) eluting the AMIGO receptor molecules from the immobilized AMIGO to which they are adsorbed with an elution buffer. In a particularly preferred embodiment of affinity purification, AMIGO is covalently attaching to an inert and porous matrix or resin (e.g., agarose reacted with cyanogen bromide). Especially preferred is an AMIGO immunoadhesin immobilized on a protein-A column. A solution containing AMIGO receptor is then passed through the chromatographic material. The AMIGO receptor adsorbs to the column and is subsequently released by changing the elution conditions (e.g. by changing pH or ionic strength).

The preferred technique for identifying molecules which bind to the AMIGO utilizes a chimeric AMIGO (e.g., epitope-tagged AMIGO or AMIGO immunoadhesin) attached to a solid phase, such as the well of an assay plate. The binding of the candidate molecules, which are optionally labelled (e.g., radiolabeled), to the immobilized AMIGO can be measured.

PRODUCTION OF TRANSGENIC ANIMALS

Nucleic acids which encode AMIGO, preferably from non-human species, such as murine or rat protein, can be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic, stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, the human

and/or mouse cDNA encoding AMIGO, or an appropriate sequence thereof, can be used to clone genomic DNA encoding AMIGO in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding AMIGO. Methods for generating transgenic animals, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for AMIGO transgene incorporation with tissue-specific enhancers, which could result in desired effect of treatment. Transgenic animals that include a copy of a transgene encoding AMIGO introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding AMIGO. Such animals can be used as tester animals for reagents thought to confer protection from, for example, diseases related to AMIGO. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the disease, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the disease.

It is now well-established that transgenes are expressed more efficiently if they contain introns at the 5' end, and if these are the naturally occurring introns (Brinster et al. Proc. Natl. Acad. Sci. USA 85:836-840 (1988); Yokode et al., Science 250:1273-1275 (1990)).

Transgenic offspring are identified by demonstrating incorporation of the microinjected transgene into their genomes, preferably by preparing DNA from short sections of tail and analyzing by Southern blotting for presence of the transgene ("Tail Blots"). A preferred probe is a segment of a transgene fusion construct that is uniquely present in the transgene and not in the mouse genome. Alternatively, substitution of a natural sequence of codons in the transgene with a different sequence that still encodes the same peptide yields a unique region identifiable in DNA and RNA analysis. Transgenic "founder" mice identified in this fashion are bred with normal mice to yield heterozygotes, which are backcrossed to create a line of transgenic mice. Tail blots of each mouse from each generation are examined until the strain is established and homozygous. Each successfully created founder mouse and its strain vary from other strains in the location and copy number of transgenes inserted into the mouse genome, and hence have widely varying levels of transgene expression. Selected animals from each established line are sacrificed at 2 months of age and the expression of the transgene is analyzed by Northern blotting of RNA from liver, muscle, fat, kidney,

brain, lung, heart, spleen, gonad, adrenal and intestine.

PRODUCTION OF "KNOCK OUT" ANIMALS

Alternatively, the non-human homologs of AMIGO can be used to construct an AMIGO "knock out" animal, i.e., having a defective or altered gene encoding AMIGO, as a result of homologous recombination between the endogenous AMIGO gene and an altered genomic AMIGO DNA introduced into an embryonic cell of the animal. For example, murine AMIGO cDNA can be used to clone genomic AMIGO DNA in accordance with established techniques. A portion of the genomic AMIGO DNA can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas and Capecchi, Cell 51:503 (1987) for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected (see e.g., Li et al., Cell 69:915 (1992)). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g., Bradley, in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harbouring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for their ability to mimic human neurological disorders and defects.

EQUIVALENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims that follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the

claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims.

EXPERIMENTAL SECTION

Materials and Methods

Ordered Differential Display

Ordered Differential Display was performed as described by Matz et al. (1997) comparing genes induced on amphoterin versus laminin matrix. Hippocampi were dissected from 18-d-old rat embryos and triturated with pasteur pipette in Hank's balanced salt solution (HBSS w/o Ca & Mg, GIBCO BRL) containing 1mM sodium pyruvate and 10 mM Hepes, pH 7.4. After washing in HBSS, neurons were suspended in Neurobasal medium (GIBCO BRL), 2% B27 supplement (GIBCO BRL), 25 μM L-glutamic acid (Sigma-Aldrich), and 1% L-glutamine (GIBCO BRL) and they were then seeded at the density of 10^6 cells on 35 mm plastic plates (Greiner) coated with laminin (10 μg/ml; Sigma-Aldrich) or recombinant amphoterin (10 μg/ml). RNA was isolated by using RNeasy mini kit (Qiagen) 24 hours after seeding and was used for ordered differential display.

Cloning of the AMIGO, AMIGO2 and AMIGO3 cDNAs

The rat AMIGO cDNA 5'end was amplificated by using method of Matz et al. (1999) based on template-switching effect and step-out PCR, and the full-length cDNA was cloned from postnatal day 14 rat cerebrum using RT-reaction with the following primers: 5'primer ACTGCTTCTCGCCTGGCCCGT; and 3'primer GAACCTCCCCATCAGCCTATACTG. The rat AMIGO sequence was used to find out human and mouse ESTs to get sequences for cloning of the human and mouse AMIGOs. The human AMIGO cDNA was cloned from the THP-1 cell-line (ATCC #TIB-202) using an RT-reaction with the following primers: 5'primer CAGAACATGCCCGGGTGAC; and 3'primer GGACCAATTCCCTTGAGGTCAG. The mouse AMIGO cDNA was cloned from adult mouse cerebrum using an RT-reaction with the following primers: 5'primer ACTGCTTCTCGCCTGGCCCGT; and 3'primer AACCTCCCCATCAGCCTACGCTG. The AMIGO sequences were used for homology search with BLAST to find possible other

related sequences. The human AMIGO2 cDNA was cloned from the HT1080 cell line (ATCC #CCL-121) as above: the 5′primer was CTCAGAGGCGACCATAATGTC and the 3′primer was TGTTTATTTTGCAGACCACACAC. The mouse AMIGO2 cDNA was cloned from adult mouse cerebrum with the following primers: 5′primer CTCAGAGGCGACCATAATGTC; and 3′primer GCGATGCTGAAGGCTAAGATG. The human AMIGO3 cDNA was cloned from the HEK293 cell line (ATCC #CRL-1573) with the 5′primer CAACCTGCACAGAGCTGCTCTGTAC and the 3′primer GCACAGTGCTTCCCACCAGTATCTG. The mouse AMIGO3 cDNA was cloned from adult mouse cerebellum with the 5′primer AGAAGTAGGTGAGTCTTGGAGCT and the 3′primer TGTTGTGCAGGTAGAGCCTG.

RT-PCR and In Situ Hybridization

Total RNA was reverse transcribed in a reaction containing 1μg RNA, 0.25mM dNTP-mix, 1μg random nonamers, 20U recombinant Rnasin (Promega), 200U MMLV-RT (Promega) with 1× MMLV reaction buffer supplied. 2μl of the reverse transcription mixture was then used for polymerase chain reaction with gene specific primers. For the mouse AMIGO the primers where as follows: 5′primer AGCAACATCCTCAGCTGCTC; and 3′primer CTTCAGCTTGTTGGAGGACAG. For mouse AMIGO2 the primers were: 5′primer GGCACTTTAGCTCCGTGATG; and 3′primer GTCTCGTTTAACAGCCGCTG. For the mouse AMIGO3 the primers were: 5′primer AGGTGTCAGAGTCCCGAGTG; and 3′primer GTAGAGCAACACCAGCACCA. For GAPDH control the primers were: 5′primer CAACGACCCCTTCATTGACC; and 3′ primer AGTGATGGCATGGACTGTGG.

The subsequent PCR reaction was performed in a PCR mix (2.5 μM dNTP, 10 mM Tris-HCL, pH 8.8, 150 mM KCL, 1.5 mM MgCl2, 0.1% Triton X-100) containing 0.2 μM 5′primer and 3′primer and 1 unit of DYNAzyme II DNA Polymerase (Finnzymes). The amplification products were separated on 1.5% agarose gel and stained with EtBr.

For in situ hybridization with radiolabeled probes, a 1.2-kb fragment from the mouse AMIGO cDNA was PCR amplified with the following primers: 5′primer CCGCTCGAGCCGGCCGATCTGTGGTTAG; and 3′primer CGGAATTCTCACACCACAATGGGTCTATCAGA. The reaction product was then ligated into pGEM-T vector. In situ hybridization analysis was carried out using single-

stranded RNA probes on mouse fetal and adult paraffin embedded tissue sections as described previously (Reponen et al., 1994).

Production of AMIGO Ig-fusion Protein

A 1180-bp BamHI fragment containing the entire extracellular coding region of the mouse AMIGO was amplified by PCR with the following primers: 5´primer CGGGATCCTAGGGTGACTCTCTCCCAGATCC; and 3´primer CGGGATCCGTTGAGGGTGTCATGGTGTCC. The reaction product was then ligated into pRMHA3-3c-FC-cDNA. The AMIGO Ig-fusion protein plasmid was cotransfected with the hygromycin resistance plasmid p-COP-hyg into Drosophila S2-cells by using the Fugene6 transfection reagent (ROCHE). After a three weeks selection with 300 µg/ml hygromycin B (Calbiochem), stabile AMIGO Ig-fusion S2-cell pools were cultured in shake flasks where the protein expression was induced with 500 µM $CuSO_4$. After culturing for 6 days the AMIGO Ig-fusion protein was isolated from the supernatant by using protein-A agarose (Upstate) according to the manufacturer's instructions.

Antibodies, Western Blotting and Immunohistochemistry

Rabbit anti-AMIGO peptide antibodies were raised against the synthetic peptide YAMGETFNET (corresponding to amino acids 341–350 of the mouse AMIGO and 342-351 of the rat and human AMIGO). Binding of the antibodies to AMIGO was verified using the recombinant AMIGO Ig-fusion protein and crude brain extracts in Western blotting (see below). Since the antibodies bound more intensely and specifically to the rat AMIGO compared to AMIGO from other species (possibly due to species differences in the glycosylation site close to the peptide sequence used in immunization), rat samples were primarily used in immunochemical detections.

Brains of embryonic, postnatal and adult rats were extracted to the final concentration of 83.3 mg tissue/ml SDS-extraction buffer (62,5 mM Tris, 1,8 % SDS, 7,75 % glycerol, 4,4 % 2-mercaptoethanol, pH 6.8). After addition of the SDS buffer, the extracts were pressed several times through a needle. The extracts were boiled 2 × 5 min and centrifuged at 10 000 × g for 10 min to remove nonsoluble material. Samples corresponding to the same wet weight of tissue were analysed by Western blotting. Ponceau staining of the membrane confirmed uniform protein amounts.

Precast 4-15% gels (Bio-Rad) were used for SDS-PAGE in Western blotting. Proteins were transferred to Hybond™ nitrocellulose membrane (Amersham Pharmacia Biotech) by Semi-dry blotting technique. Rabbit anti-AMIGO peptide antibody (1/1000 dilution) and monoclonal anti-CNPase, clone 11-5B (Sigma, 1/1500) were used as primary antibodies. HRP-conjugated goat anti-rabbit IgG (Bio-Rad) and sheep anti-mouse IgG (AP Biotech) were used as secondary antibodies. The antibody comples were detected using ECL™ reagents (AP Biotech).

Immunohistochemistry of AMIGO was performed using paraffine sections. In brief, adult rats were sacrificed after CO_2 treatment by cervical dislocation and tissues were fixed by using ice-cold PBS with 4% paraformaldehyde, and the samples were then transferred in paraffine. Hydrated paraffine sections (4–10 μm thick) were incubated with 1% hydrogen peroxide/methanol solution for 20 min, and washed again with PBS. The sections were blocked for 1h with 5% skimmed milk powder in PBS. The sections were then incubated with the rabbit AMIGO peptide antiserum, which was diluted 1/200 in the blocking buffer at +4 °C overnight. After washing with PBS, the sections were incubated with HRP conjugated goat anti–rabbit antibodies (Biorad) at a dilution of 1:500 for 2 h at room temperature, washed with PBS and incubated with aminoethyl carbazole (AEC, Sigma) as a chromogenic substrate. Immunofluorescence staining for in vitro cultured hippocampal neurons was performed by using FITC conjucated goat anti-rabbit secondary antibodies (Jackson lab).

Neurite Outgrowth Assay

Hippocampi were dissected from 18-day-old rat embryos into a Ca-Mg-free trituration medium (HBSS with 1mM sodium pyruvate and 10 mM HEPES, pH 7.4). Cells were dissociated by pipetting 25 times with glass pasteur pipette and washed once with the Ca-Mg-containing buffer (HBSS+Ca+Mg with 1mM sodium pyruvate and 10 mM HEPES, pH 7.4). The cells were seeded at the density of 70000 cells/cm^2 on 96-well polystyrene dishes coated by the test protein in Neurobasal medium with 2% B27 supplement (GIBCO BRL), 1% BSA, 0.5 mM L-glutamine, 25μM L-glutamic acid and 1X penicillin-streptomycin. The dishes were coated with the test protein (3.125-100 μg/ml) in PBS overnight at 4°C, washed tree times with PBS, and blocked with 1%BSA in PBS for 1 h at room temperature before

adding the cells. The cells were cultured for 24 h before counting the neurite outgrowth. For counting of neurite outgrowth, images were taken from living cells using randomly selected microscopic fields and the extensions, which were twice the length of the cell soma, were considered as neurites. For quantification of neurite outgrowth, 15 images (275μm x 225μm) with a total of 750 cells were evaluated from every concentration of the test protein (AMIGO Ig-fusion or Fc control substrate) used for coating. The data were pooled from three independent experiments.

To test the effect of soluble AMIGO Ig-fusion protein the dishes were coated with the AMIGO Ig-fusion protein (12.5 μg/ml in PBS) at 4°C overnight, washed three times with PBS, and blocked with 1%BSA in PBS for 1 h at room temperature. The cells were seeded at the density of 70000 cells/cm^2 and cultured for 24 h before counting the neurite outgrowth. Counting was carried out as above from three independent experiments. A total of 750 cells were evaluated for every concentration of the test protein (AMIGO Ig-fusion or the Fc control protein) used in solution.

In vitro fasciculation Assay

Fasciculation of neurites was studied with hippocampal neurons prepared as above. The 96-well plates were coated with poly-L-lysine at +4°C overnight, washed three times with PBS, and blocked with 1%BSA in PBS for 1 h at room temperature. The cells were seeded at the density of 70000 cells/cm^2 in the serum free medium (see "Neurite outgrowth assay") with either the AMIGO Ig-fusion protein or the Fc control protein in solution. The AMIGO Ig-fusion and the Fc control protein were tested at 3.25-25 μg/ml. The experiment was repeated independently 3 times, and pictures were taken from living cells after 4 days in culture. For quantification of neurite outgrowth, 12 randomly taken images (45 μm x 35 μm) were taken for every concentration of the AMIGO Ig-fusion and the Fc control protein used in solution. To evaluate inhibition of fasciculation, the total length of the processes, the diameter of which is < 2 μm (formed only from 1-3 neurites), was measured from the 12 images taken for every protein concentration tested.

Pictures for the neurite outgrowth and fasciculation experiments were taken with Olympus DP10 digital camera. The measurements were carried out by using the Image-Pro image analysis software.

Binding Assays

Coimmunoprecipitation experiments were performed using transiently transfected HEK293T cells. The constructs were transfected into the cells by using FUGENE6 (ROCHE) according to the manufacturer's instructions. The full length AMIGO was cloned in frame with the pEGFP-N1 (Clontech) and pcDNA6-V5-His (Invitrogen) vectors. The full length RAGE was cloned in frame with the pcDNA6-V5-His vector. After transfection, the cells were grown for 48 h before lysing in the RIPA buffer with 10 mg/ml PMSF and 60 μg/ml aprotinin (SIGMA). Coimmunoprecipitation experiments were carried out using rabbit anti-GFP antibody (Santa Cruz; sc-8334) and mouse anti-V5 antibody (Invitrogen; 46-0705) at the concentration of 1 μg/ml.

The aggregation assay was carried out using protein A Fluoresbrite carboxylated beads (Polysciences, size 1μm). The beads (100 μg) were first washed 3 times with PBS, 2%BSA, 0.1% Tween-20 solution and they were the mixed and sonicated in water bath in 50 μl of the buffer mentioned above. The beads were divided to two aliquots, and the test and the control protein (10 μg each) were added into the beads in 25 μl of PBS, 2%BSA and 0.1% Tween-20 solution (final volume 50 μl). After addition of the protein 2 μl samples were taken into 100 μl of PBS, 2%BSA, 0.1% Tween-20 solution in 96-well plate at different time points. The plate was incubated at room temperature and the aggregation was evaluated under the fluorescence microscope. Kinetics of bead aggregation was calculated from three independent experiments from 12 fields containing 1500 beads. The extent of bead aggregation is represented by the index N_t/N_0 where N_t and N_0 are the total number of particles at the incubation times t and 0 (Agarwala et al., 2001).

Coimmunoprecipitation of AMIGO and AMIGO2 with EGFR

Coimmunoprecipitation experiments were performed using stable HEK293 cells expressing EGFR. The constructs were transfected into the cells by using FUGENE6 (ROCHE) according to the manufacturer's instructions. The full length and extracellular part (EC-part) AMIGO, AMIGO2 and AMIGO3 were cloned in frame pcDNA6-V5-His (Invitrogen) vectors. After transfection, the cells were grown for 48 h before lysing in the RIPA buffer with 10 mg/ml PMSF, 60 μg/ml aprotinin (SIGMA) and 1 mM EDTA. Coimmunoprecipitation experiments were carried out using rabbit anti-EGFR antibody

(Santa Cruz) and mouse anti-V5 antibody (Invitrogen; 46-0705) at the concentration of 1 µg/ml.

EGFR phosphorylation experiment

EGFR phoshorylation experiments were performed using HEK293T cells. The constructs were transfected into the cells by using FUGENE6 (ROCHE) according to the manufacturer's instructions. The full length AMIGO, AMIGO2 and AMIGO3 were cloned in frame with pcDNA6-V5-His vector (Invitrogen). The full length human EGFR was cloned with C-terminal Flag-tag into pcDNA6 vector (Invitrogen). The cells on 50 % confluent 6 cm plate were transfected with 0.3 µg of EGFR plasmid and with 1.7 µg of AMIGO, AMIGO2, AMIGO3 or control plasmid (pcDNA6-V5-His, Invitrogen). After 24 hours of transfection cells were starved for 4 hours without serum. The autophosphorylation of the EGFR was induced by adding 50 ng/ml of EGF for 5 minutes in +37°C. The cells were lysed and immunoprecipitated with anti-phospho-Tyrosine antibody (clone PY20). The cells were also immunoprecipitated with anti-Flag-tag antibody (clone M2). The samples from anti-phospho-Tyrosine immunoprecipitation were detected on western blot by using the anti-flag-tag antibody to see the EGFR phosphorylation differences between the samples. The samples from anti-Flag-tag immunoprecipitation were detected on western blot by using the anti-phospho-Tyrosine antibody to see the EGFR phosphorylation differences between the samples.

Homo- and heterophilic binding of AMIGO, AMIGO2 and AMIGO3

Coimmunoprecipitation experiments were performed using transiently transfected HEK293T cells. The constructs were transfected into the cells by using FUGENE6 (ROCHE) according to the manufacturer's instructions. The full length and extracellular part (EC-part) AMIGO, AMIGO2 and AMIGO3 were cloned in frame with the pEGFP-N1 (Clontech) or pcDNA6-V5-His (Invitrogen) vectors. The full length RAGE was cloned in frame with the pcDNA6-V5-His vector. After transfection, the cells were grown for 48 h before lysing in the RIPA buffer with 10 mg/ml PMSF and 60 µg/ml aprotinin (SIGMA). Coimmunoprecipitation experiments were carried out using rabbit anti-GFP antibody (Santa Cruz; sc-8334) and mouse anti-V5 antibody (Invitrogen; 46-0705) at the concentration of 1 µg/ml.

Knockout constructs for AMIGO, AMIGO2 and AMIGO3.

For AMIGO gene targeting, we constructed a replacement vector by using genomic DNA fracments from mouse phage library (strain 129SV). The whole coding region of AMIGO gene was replaced by inserting Beta-galactosidase gene under the promoter of AMIGO gene using tailored PCR primers: 5´primer GCGGCCGCTCAGGGCCCACGGTTTCTGCAG (with NotI site) and 3´primer GGCGCGCCACTGGGAAGAGVGAGGAAGGCCAC (with AscI site). For positive selection, we cloned the neomycin-resistance gene after the beta-galactosidase gene. The 3´prime homologous arm was inserted into the vector as a KpnI/NcoI fragment (NcoI blunted). The length of the homologous recombination arms where 9.9 kb for 5´arm and 2.0 kb for 3´arm.

For AMIGO2 gene targeting, we constructed a replacement vector by using genomic DNA fracments from mouse phage library (strain 129SV). The whole coding region of AMIGO2 gene was replaced by inserting human placental alkaline phosphatase gene under the promoter of AMIGO2 gene using tailored PCR primers: 5`primer TAAACTAGCGGCCGCTCATGGAGGCTCACCCATGGAC (with NotI site) and 3´primer AGATATGGCGCGCCGGTCGCCTCTGAGTCTCTTGCCAG (with AscI site). For positive selection, we cloned the neomycin-resistance gene after the human placental alkaline phosphatase gene. The 3´ homologous arm was inserted into the vector as a BamHI/HindIII fragment (HindIII blunted). The length of the homologous recombination arms where 3.0 kb for 5´arm and 3.0 kb for 3´arm.

For AMIGO3 gene targeting, we constructed a replacement vector by using genomic DNA fracments from mouse phage library (strain 129SV). The whole coding region of AMIGO3 gene was replaced by inserting EGFP gene under the promoter of AMIGO3 gene using tailored PCR primers: 5`primer ACCTTAATTAACCAGATGGCTTCTTCTTTC (with PacI site) and 3´primer AGATATGGCGCGCCAGTGACTACCAGGGAAGAT (with AscI site). For positive selection, we cloned the neomycin-resistance gene after the EGFP gene. The 3´ homologous arm was inserted into the vector as a BamHI fragment. The length of the homologous recombination arms where 3.5 kb for 5´arm and 2.6 kb for 3´arm.

Using standard procedures, we electroporated R1 mouse embryonic stem cells, suspenced in PBS, with 20 μg linearized (AMIGO:NotI, AMIGO2:NotI and AMIGO3: PacI) targeting vector, using BioRad Gene Pulser (240 V and 500 μF). Transfected cells were selected with 300 μg/ml G418 (Gibco). On day 9-11 after electroporation, we picked 100-400 clones and identified resistant clones with homologous recombination by PCR amplification using primers for neomycin resistance gene and outside the targeted locus. PCR results were confirmed by using southern blots with probes outside the targeting locus.

Using standard procedures, selected embryonic stem cells were aggregated into ICR morulas and aggregates were transferred to pseudopregnant foster mothers. Highly chimeric males were bred to ICR females and heterozycos offsprings were intercrossed to obtain homozygous mutant mice. For genotyping the genomic DNA was isolated from tail biopsies with protein K digestion and isopropanol precipitation. For routing genotyping, we used PCR where first reaction contains oligos which could amplificate product only from intact AMIGO, AMIGO2 or AMIGO3 gene locus (from inside the genes). The second PCR reaction contains oligos which could only amplificate product from targeted locus (one oligo from neomycin gene and the second from 3′ homologous arm used for targeting).

These AMIGO, AMIGO2 and AMIGO3 single knockout mice strains have been used to generate double knockout mice strains (ΔAMIGO/ΔAMIGO2; ΔAMIGO/ΔAMIGO3; ΔAMIGO2/ΔAMIGO3) and triple knockout mouse strain (ΔAMIGO/ΔAMIGO2/ΔAMIGO3) by using standard breeding procedures. The genotype of the mutant mice were confirmed by using same PCR reactions as in single knockout strains.

AMIGO ig-fusion transgenic animals

The DNA region encoding mouse AMIGO extracellular part was amplified by PCR from mouse AMIGO cDNA using the BamHI-containing upstream primer CGGGATCCTAGGGTGACTCTCTCCCAGATCC and the BamHI-containing downstream primer CGGGATCCGTTGAGGGTGTCATGGTGTCC. PCR fragment was cloned into frame with human IgG FC-part in expression vector pRMHA3-3c-FC. The

DNA region encoding mouse AMIGO extracellular part fused with IgG FC-part was amplified by PCR using the NotI-containing upstream primer ATAAGAATGCGGCCGCCAATGTGCATCAGTTGTGGTCAG and the XbaI-containing downstream primer GCTCTAGACGTGCCAAGCATCCTCGTGCGAC. The PCR fragment was cloned into a vector psisGI. In the resulting plasmid, the open reading frame of AMIGO ig-fusion was located under the control of a PDGF-beta promoter and supplied with the polyadenylation signal of the bovine growth hormone. The construct was injected into the pronuclei of oocytes from superovulated females of C57BL/6 strain. The transgene integration was determined by Southern blot and PCR analyses of tail DNA. To establish the transgenic line, founders were crossed with C57BL/6 animals.

Regeneration experiment with AMIGO, AMIGO2 or AMIGO3 proteins.

Spinal cord injury and delivery of AMIGO, AMIGO2 or AMIGO3 can be made as follows. BALB-c female mice (n = 70) are anesthetized with 0.4 ml/kg hypnorm and 5 mg/kg diazepam. A segment of the thoracic spinal cord is exposed using fine rongeurs to remove the bone, and a dorsal over-hemisection was made at T7. Fine scissors are used to cut the dorsal part of the spinal cord, which is cut a second time with a fine knife to ensure that the lesion extends past the central canal. The SABER Delivery System (DURECT Corporation) is according to manufacturer's instructions AMIGO, AMIGO2 or AMIGO3 Ig-fusion proteins are added into the SABER solution in concentration of 1-100 mg/ml. As controls, a second group of animals receives SABRE solution with PBS buffer, and a third group is left untreated. For retransections 3 weeks after SCI, the spinal cords are cut at T6 as described above, and the animals are tested using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale on days 1, 2, and 6 after the second surgery. Alternatively, Ig-fusion protein is replaced with AMIGO ectodomain as described below.

Axonal regeneration experiment with soluble AMIGO, AMIGO2 or AMIGO3 ectodomains.

Spinal cord dorsal hemisection and corticospinal fiber tracing is adapted from GrandPre et al. (2002) Nogo-66 receptor antagonist peptide promotes axonal regeneration. Nature 417: 547–551. Adult female C57BL/6 mice (8–10 weeks of age) are deeply anesthetized with intramuscular ketamine (100 mg/kg) and intraperitoneal xylazine (15 mg/kg). A complete laminectomy is performed, and the dorsal part of spinal cord is fully exposed at levels T6 and T7. The dorsal half of the spinal cord is cut with a pair of microscissors to sever the

dorsal parts of the corticospinal tracts, and the depth of lesion (approximately 1.0 mm) is assured by passing the sharp part of a number 11 blade across the dorsal half of the cord. An osmotic minipump (Alzet model 2002, Alza, Mountain View, CA) is implanted after the hemisection of dorsal spinal cord and positioned to deliver reagents to the subcutaneous space. A catheter connected to the outlet of the minipump is inserted into the intrathecal space of the spinal cord at the T7 level through a small hole in the dura. The pump is filled with vehicle (97.5% PBS plus 2.5%DMSO) or soluble AMIGO, AMIGO2 and/or AMIGO3 ectodomain in the vehicle. The vehicle or soluble AMIGO, AMIGO2 and/or AMIGO3 are delivered continuously at a rate of approx 0.6 µl/hr for 14 d and the soluble AMIGO, AMIGO2 and/or AMIGO3 ectodomain doses are 2.0, 7.5 and 15.0 mg · kg-1 · d-1. For those mice receiving soluble AMIGO, AMIGO2 and/or AMIGO3 ectodomain without spinal cord injury, the laminectomy and minipump placement are accomplished in the same fashion. Two weeks after lesion, a burr hole is made on each side of the skull overlying the sensorimotor cortex of the lower limbs. The anterograde neuronal tracer biotin dextran amine (BDA, 10% in PBS) is applied at four injection sites at a depth of 0.5-0.8 mm from the cortical surface on each side. Two weeks after BDA injection, the animals are killed by perfusion with PBS, followed by 4% paraformadehyde. The spinal cord extending from 6 mm rostral to 6 mm caudal from the lesion site is cut parasaggitally (50 µm) on a vibrating microtome. Transverse sections are collected from the spinal cord 8–12 mm rostral to and 8–12 mm caudal to the injury site. The sections are incubated with avidin-biotin-peroxidase complex and the BDA tracer for regenerated axons is visualized by nickel-enhanced diaminobenzidine HRP reaction. For bevioral analysis vehicle-treated and soluble AMIGO, AMIGO2 or AMIGO3 ectodomain treated mice are compared using the Basso-Beattie-Bresnahan (BBB) locomotor rating scale according to Basso et al (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12, 1-21.

Inhibition of glial scar formation in CNS with soluble AMIGO, AMIGO2 and AMIGO3 proteins

Stereotactic lesioning of the cerebral cortex and intraventricular cannulation can be made according to (Logan et al., 1994). Adult female 200- to 250-g Wistar rats are assigned to two treatment groups of 5 animals each receiving: (i) 30 µg/10µl/day Fc-control protein ;

or (ii) 30 μg/10μl/day AMIGO, AMIGO2 and/or AMIGO3 Ig-fusion protein in saline. On day 0 of the experiment, a stereotactically defined unilateral incisional lesion is placed through the cerebral cortex into the lateral ventricle at the same time as ipsilateral placement of a permanent intraventricular cannula. Reagents (10 μl) are perfused into the lesion site by daily intraventricular injections through the cannulae for 14 days under halothane anaesthesia. After 14 days post lesion (dpl), animals are killed and their brains processed for immunohistochemical analysis of the lesion site. Alternatively, Fc-fusion protein is replaced with AMIGO ectodomain in order to avoid immune response against Fc domain, hence the treatment groups of 5 animals are comprise: (i) 10μl/day phosphate buffered saline (PBS); or (ii) 30 μg/10μl/day soluble AMIGO, AMIGO2 or AMIGO3 ectodomain in phosphate buffered saline.

Modulation of tumour metastasis by using soluble AMIGO, AMIGO2 or AMIGO3 extracellular domain.

The modulation of tumour metastases assay can be performed as follows. Lewis lung murine carcinoma cells are injected into the dorsal midline of male, 6–8-week-old C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME). Primary tumours are surgically excised when tumour volume is 1,500 mm^3 (day 14). For three days before the removal of primary tumor, mice receive AMIGO-, AMIGO2- or AMIGO3 Ig-fusion protein or control FC-part protein once daily, 21 days after removal of primary tumour. Weight of the lungs and numbers of lung surface metastases are determined under X4 magnification using an Olympus microscope after intratracheal injection of India Ink (15%). Alternatively, animal experiments are adapted from Liao et al. (2000). For the pulmonary metastasis model, C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) are injected intra-footpad with 1 x 10^5 cells of murine Lewis lung carcinoma. When footpad tumors reach 5 mm in diameter, the tumor-bearing leg is surgically ligated. Mice are then divided into two groups receiving injections of approx 20-30 mg/kg/dose of either vehicle (phosphate buffered saline) or vehicle with soluble AMIGO, AMIGO2 or AMIGO3 ectodomain every 3 days for 3 weeks. Weight of the lungs and numbers of lung surface metastases are determined under X4 magnification using an Olympus microscope after intratracheal injection of India Ink (15%).

Blockage of local tumour growth with soluble AMIGO, AMIGO2 or AMIGO3 extracellular domain.

Rat C6 glioma cells are injected into the dorsal midline of female NCR immunocompromised mice aged 4–6 weeks (Taconic Farms, Germantown, NY). Alternatively, rat C6 glioma cells are injected into the dorsal midline of female mice with severe combined immunodeficiency (SCID; Taconic Farms). Administration of AMIGO-, AMIGO2- or AMIGO3 Ig-fusion protein or control FC-part protein is done once daily to immunocompromised (athymic nude) mice upon injection of rat C6 glioma cells. Tumours are measured at day 21 with calipers and the volume is calculates: $V = \pi*h(h^2 + 3a^2)/6$, where h = height of the tumour segment; a = (length + width of the tumour)/4; and V = volume of the tumour. Tumour tissue is retrieved, fixed in formalin (10%) and paraffin-embedded sections are prepared. Alternatively, Human A431 squamous cell carcinoma xenografts are established in athymic nude nu/nu mice, 6-8 weeks of age through subcutaneous inoculation of 0.5-2 $*10^6$ cells into the dorsal flank of each mouse. Administration of AMIGO-, AMIGO2- or AMIGO3 Ig-fusion protein or control FC-part protein (approx 10-40 mg/kg/dose) is done once daily to immunocompromised (athymic nude) mice upon injection of human A431 squamous cells. Tumours are measured at day 21 with calipers and the volume is calculates: $V = \pi*h(h^2 + 3a^2)/6$, where h = height of the tumour segment; a = (length + width of the tumour)/4; and V = volume of the tumour. Tumour tissue is retrieved, fixed in formalin (10%) and paraffin-embedded sections are prepared.

Suppression of tumorigenicity by lentivirus-mediated gene transfer of soluble or full length AMIGO, AMIGO2 or AMIGO3

Animal experiments are adapted from Reed et al. (2002) Suppression of tumorigenicity by adenovirus-mediated gene transfer of decorin. *Oncogene* 21:3688-95. Human WiDr colon and A431 squamous cell carcinoma xenografts are established in athymic nude nu/nu mice, 6-8 weeks of age through subcutaneous inoculation of 0.5-2 $*10^6$ cells into the dorsal flank of each mouse. Mice are carefully examined every 2 or 3 days and any tumor growth is measured with a micro-caliper according to the following formula: V=a (b2/2), where a and b represent the larger and smaller diameters, respectively. When tumors reach 2-3 mm in greater diameter, each mouse receives direct intra-neoplastic injections and also three

other injections 2 ,4 and 6 days after first injection. The injections contain (approx 50 µl containing 4 * 10^7 TU) replication-incompetent lentivirus, either empty virus or virus harboring the full-length AMIGO, AMIGO2 or AMIGO3 or soluble AMIGO, AMIGO2 or AMIGO3 ectodomain gene. Student's two-sided t-test is used to compare the values of the treated and control samples. A value of $P<0.05$ is considered as significant.

Animals are sacrificed at the end of the experiments, between 19 and 58 days depending on the treatment regimen and inoculum size, and each tumor is carefully dissected. The tumors are fixed in 10% buffered formaldehyde, embedded in paraffin and processed for routine histology. To determine the proliferative index of tumor xenografts, the percentage of tumor cell nuclei positive for Ki-67 marker is estimated in 10 high-power (´400) fields per animal.

Results

Idenfication and Cloning of a Novel Family of Transmembrane Proteins Containing a Tandem Array of Leucine-rich Repeats and an Immunoglobulin Domain (AMIGO, AMIGO2 and AMIGO3)

Ordered differential display (ODD; Matz et al., 1997) was used to search for amphoterin-induced genes in neurons. Comparison of ODD from embryonic day 18 rat hippocampal neurons grown on amphoterin and laminin coated plates revealed a transcript that was expressed more on amphoterin (Fig. 1 A). This expression difference was also confirmed with RT-PCR (Fig. 1 B).

The sequence of the partial transcript did not give homology with any previously cloned genes. By using the 5´RACE method (Matz et al., 1999) the cDNA encoding the whole coding sequence was cloned (Fig. 2 A). We named this differentially expressed gene as AMIGO (AMphoterin Induced Gene and Orphan receptor). Hydrophobicity profile analysis (Nielsen et al., 1997; software SignalIP V2.0.b2) revealed that the protein sequence of AMIGO contains a putative signal sequence and a putative transmembrane region. The deduced extracellular part of the protein contains six leucine-rich repeats (LRRs) and one immunoglobulin domain. The deduced cytosolic part of the protein does not contain any known domains.

The human and mouse counterparts of AMIGO were also cloned with the 5'RACE method by using data from the rat AMIGO sequence and from EST sequences. Identity at the amino acid level between the rat and mouse AMIGO is 95% and the murine sequences are 89% identical to the human AMIGO. In the extracellular part the most conserved motifs between the murine and human AMIGO are the N-terminal cysteine-rich domain and the LRRs 1-3. Interestingly, the whole transmembrane domain and the cytoplasmic tail are 100% identical between the murine and human AMIGO.

By using homology search we detected ESTs which gave homology but were not identical as compared to AMIGO. By using these EST sequences we cloned two other novel proteins which we named for convenience as AMIGO2 and AMIGO3. The deduced amino acid sequences show that AMIGO2 and AMIGO3 have the same domain organization as AMIGO: they also contain a putative signal seguence for secretion and six LRRs flanked on both the N and C-terminal sides by cysteine-rich LRRNT and LRRCT-domains. Like AMIGO, the deduced extracellular parts of AMIGO2 and AMIGO3 contain an immunoglobulin domain close to the transmembrane domain (for schematic picture of AMIGO, -2 and –3, see Fig. 2 B).

Similarity at the amino acid level between AMIGO to AMIGO2 is 48%, AMIGO to AMIGO3 is 50% and AMIGO2 to AMIGO3 is 48%. The alignment for AMIGO, -2 and -3 shows that the most conserved regions between the three proteins are the LRRs, the transmembrane region and some parts of the cytosolic tail (Fig. 2 A). The LRRs found in the AMIGOs can be described as a motif $LX_2LXLX_2NX(L/I)X_2aX_4(F/L/I)$(in which "a" denotes an aliphatic residue and "X" any amino acid); this motif resembles a typical LRR sequence often found in extracellular parts of animal proteins (Kajava, 1998).

Expression of the Gene Family Members in Adult Tissues

RT-PCR analysis of adult mouse tissues (Fig. 3) revealed that AMIGO is mainly expressed in the nervous tissues (cerebellum, cerebrum and retina) although some low expression could be also seen in liver, kidney, small intestine, spleen, lung and heart. AMIGO2 expression is most prominent in cerebellum, retina, liver and lung. A lower AMIGO2 mRNA expression is also seen in cerebrum, kidney, small intestine, spleen and testis. AMIGO3 mRNA expression could be detected in every tissue studied showing no specific

expression pattern compared to AMIGO or AMIGO2. It thus appears that AMIGO is essentially a nervous system specific member of the protein family and we focused on AMIGO in more detail in the present study.

Cerebrum

In adult rat cerebrum the AMIGO staining was found from many nerve fiber bundles and nerve paths (Figure 7 and 9a). When compared to anti-CNPase staining, the AMIGO staining co-localizes with almost every myelinated areas of the cerebrum. In this study the only white matter area where AMIGO staining was absent was the lateral tractus olfactorius.

However, the AMIGO expression is not restricted to myelinated tracts; for example in hippocampus, non-myelinated tracts in the stratum lucidum CA3 region, which were negative for anti-CNPase and myelin basic protein (myelin basic protein data not shown), stained clearly with anti-AMIGO (Figure 9a and c). In coronal sections staining was restricted in the stratum lucidum of the CA3-region where it was localized more precisely in basal areas of the apical dendrites of the pyramidal cells (Figure 8). The anti-AMIGO seemed to stain not the dendrites but the areas around the basal areas of the apical dendrites. In sagital sections the AMIGO staining was seen to be slightly fibrous (Figure 9c and d). The localization and structure of the AMIGO staining in hippocampus reminds the one seen for mossy-fibers. The mossy fibers are the axons of the granule cells from dentatum gyrus, which end up in the stratum lucidum of the CA3-region, where they form synapses with the apical dendrites of the pyramidal cells. The mossy-fibers have been shown to stain very intensively with anti-neurofilament antibodies (Huber et al., 1985). Our anti-NF-M staining in hippocampus was very similar when compared to anti-AMIGO staining, which supports the interpretation that AMIGO localizes in mossy-fibers or structures very closely related to them. On the other hand these structures could be the interneuronal axons of the CA3-region, which have been shown to proceed along the mossy-fibers in stratum lucidum (Vida and Frotscher, 2000).

In cerebral cortex the AMIGO immunostaining was seen only in particular regions, which were also immunoreactive for anti-CNPase and anti-NF-M (Figure 7). The cortical staining for all of the three antibodies used (AMIGO, CNPase and NF-M) was diffuse and indistinct, which is related in myelinated axons. At the same time the AMIGO staining is

seen in the basal areas of the apical dendrites of the cortical pyramidal cells but interestingly not all of the apical dendrites are AMIGO immunoreactive. The anti-NF-M staining was also found in the apical dendrites but the staining could also be seen in the cell soma and the basal dendritic areas of the pyramidal cells (Figure 10).

Cerebellum

In the cerebellum the anti-AMIGO staining was also co-localized with the anti-NF-M staining. In the cerebellum the anti-neurofilament antibodies have been seen to stain very intensively myelinated axons and basket cell axons (Matus et al., 1979).

The AMIGO staining was intensive in white matter and in the myelinated axons of the granular cell layer resembling the one seen for anti-NF-M. The most intensive staining in white matter was found in the middle of the cerebellum where the staining was seen in a string of pearls like structures (Figure 11a and b).

In the cortical areas of the cerebellum the AMIGO staining was seen in both sides of the Purkinje cell layer. The basket like structure around the Purkinje cell somas were seen to be immunoreactive for AMIGO and this structure is formed by the basket cell axons (Figure 11a and b).

In the molecular layer of the cerebellum the AMIGO staining is seen in the fibers, which are orientated along the Purkinje cell layer (Figure 11). At least some of these fibers are basket cell axons but also some other axons are AMIGO positive because the AMIGO immunostaining was more intensive when compared to anti-NF-M staining (data not shown).

Also the nuclei in the middle part of the cerebellum were AMIGO immunoreactive. In nuclei the AMIGO and NF-M staining differed form each other because AMIGO staining was only seen in neurites but NF-M staining could also be found from neurites and cell soma.

Pons and medulla oblongata

In pons and medulla oblongata the AMIGO staining was found in white matter.

Spinal cord

In the cross-sections of the spinal cord the anti-AMIGO staining was seen in the white matter as a dotted like structures. In paraffin sections the myelin sheaths have melted away leaving round holes where the myelin has been located. In these sections the AMIGO staining is seen in the dots in the middle of the holes (Figure 12a). Also the anti-NF-M antibodies stained these dots (Figure 12c) whereas the anti-CNPase did not stained the same structures (Figure 12b). In cryosections the AMIGO staining was seen to localize in the middle of the myelinated axons and not into the multilayered myelin sheaths (data not shown.). It is not clear whether all of the AMIGO positive axons were myelinated or not due to the limitations of the light microscopy.

In the grey matter of the spinal cord the anti-AMIGO stained some nerve fibers. Only some fibers of the grey matter, which were crossing into the white matter, were AMIGO positive. This suggests that AMIGO is expressed only in some subpopulation of these crossing axons (data not shown).

Kidney, optic nerve and femoral nerve

The AMIGO staining was found to co-localize with anti-NF-M staining in kidney. The stained structures were defined as autonomous nerve fibers (Figure 13). The optic nerve was intensively stained with the anti-AMIGO antibodies whereas in femoral nerve the staining was absent (data not shown).

Embryos

In the head of the E18 rat embryo the staining was seen in nerve fibers and in nerve fiber tracts of internal capsule (Figure 14 c), optic tract (Figure 14a), middle cerebellar peduncle, stria medullaris, fasciculus retroflexus and longitudinal fasciculus pons. The AMIGO positive staining co-localized with anti-NF-M but the CNPase was not immunohistochemically detectable in E18 embryo (data not shown).

In the whole sections of the E16 embryo anti-AMIGO immunostaining was found only in some parts of the developing brain area, in optic nerve and areas close to the intestine and the rib bones (data not shown).

Expression of AMIGO During Development

The AMIGO mRNA expression was studied in more detail using in situ hybridization. The AMIGO antisense probe gave a clear signal in the developing and adult nervous tissues whereas the sense probe did not give any clear signal (sense probe data not shown). A clear AMIGO expression was already detected in the E13 rodent embryo; at this stage the highest expression level was found in the dorsal root ganglia and the trigeminal ganglion with some expression in the central nervous system (Fig. 4 A-B). During later stages of development and in the adult, AMIGO was also prominently expressed in the brain, where the most intense signal was detected in the hippocampus (Fig. 4 C).

To investigate the expression of AMIGO at the protein level, polyclonal antisera were produced against an extracellular 10-amino acid peptide sequence that is found in AMIGO but not in AMIGO 2 or 3. The anti-peptide antibodies recognized the 75-kD AMIGO Ig-fusion protein produced in Drosophila S2 cells (Fig. 5, lanes 1 and 3). Western blotting of crude brain extracts revealed specific binding to a 65-kD polypeptide (Fig. 5, lanes 2 and 4). The molecular mass of the recognized polypeptide is close to the calculated molecular mass (56-kD) of AMIGO. Binding of the antibodies to both the fusion protein and the 65-kD polypeptide of brain were blocked by the synthetic peptide used as the immunogen (Fig. 5, lanes 3-6).

Western blotting of AMIGO using crude brain extracts from different developmental stages was consistent with the in situ hybridization data. The expression appears to start in the brain somewhat later than in the peripheral nervous system and increases clearly between E13 to E14 (Fig. 6). The expression is maintained high during the perinatal developmental stage but is downregulated during the postnatal stages P6 to P10. After this, the expression is again upregulated and remains high in the adult brain (Fig. 6). Since the time period of the postnatal upregulation of the AMIGO expression would appear to coincide with the onset of myelination, we compared the expression of AMIGO to that of the myelin-specific marker α-CNPase. Indeed, the expression of AMIGO and the CNPase display a parallel increase during postnatal development (Fig. 6). The AMIGO expression thus displays a dual character during brain development; the first expression peak occurs during the late embryonic and perinatal development, and the second increase in expression accompanies myelination.

Immunohistochemistry using the anti-peptide antibodies revealed specific staining only in the nervous system. In general, intensity of the immunostaining was in agreement with the expression data inferred from Western blotting (Fig. 6). Further, specificity of the immunostaining was suggested by inhibition of antibody binding to tissue sections by the peptide used as the immunogen (Fig. 5, panel B). In general, AMIGO was intensely stained in developing and mature fiber tracts. During embryonic development when the spinal ganglia express abundantly AMIGO mRNA (see Fig. 4), the immunostaining was observed in the fiber tracts connecting to the ganglia and the spinal cord but not in the ganglia themselves (Fig. 7, panel A), suggesting that the AMIGO protein is transported to axonal processes. In cerebellum, the most intense staining was observed in fibers on both sides of the Purkinje cell layer; the characteristic structure formed by the basket cell axons around the Purkinje cell soma was clearly discerned by the AMIGO immunostaining (Fig. 7 B). Consistent with the Western blotting data, AMIGO immunostaining labeled most myelinated axon tracts in the adult. An example is shown in Fig. 7 (panels C and D), demonstrating the similarity of the AMIGO and α-CNPase immunostaining around the hippocampus. However, the AMIGO expression is not restricted to myelinated tracts; for example in hippocampus, non-myelinated tracts in the stratum lucidum CA3 region, which were negative for α-CNPase (Fig. 7 D) and myelin basic protein (data not shown), stained clearly for AMIGO (Fig. 7 C). In general, AMIGO staining was detected (both during development and in adult animal) in large-diameter neurites (axons) that were also stained by antibodies against the 145 kD neurofilament (data not shown). As in the forebrain, myelinated axon tracts were also stained for AMIGO in cerebellum, pons, medulla and spinal cord.

AMIGO was also clearly immunostained both in the cell soma and in fasciculated and non-fasciculated processes of cultured hippocampal neurons (Fig. 7 F). As expected from immunostaining of tissue sections, double-immunostaining (not shown) revealed colocalization with the 145-kD neurofilament and the β-tubulin (TuJ1) but not with MAP2. AMIGO is thus preferentially expressed in axonal rather than dendritic processes.

AMIGO Promotes Neurite Extension of Hippocampal Neurons

Identification of AMIGO from hippocampal neurons growing neurites on amphoterin, the occurrence in fiber tracts in vivo and the domain structure with LRRs and Ig domains suggest that AMIGO might have a role in neurite extension. To get insight into the

function of AMIGO, we tested if it is able to promote neurite outgrowth of hippocampal neurons. The extracellular part of the AMIGO was fused to human IgG Fc part, and this fusion protein was immobilized on microtiter wells and used as a substrate for hippocampal neurons. These experiments showed that the AMIGO Ig-fusion protein promotes attachment and neurite outgrowth of hippocampal neurons (Fig. 8 A and C), whereas on the human IgG Fc control neurite outgrowth was very low or undetectable figure (Fig. 8 B and C). Neurite outgrowth induced by the immobilized AMIGO Ig-fusion protein was inhibited by the soluble AMIGO Ig-fusion in the culture medium (Fig. 8 D).

Soluble AMIGO Perturbs Development of Fasciculated Axon Tracts in Vitro

Because AMIGO immunostaining could be found in vitro in hippocampal fasciculating axons and in the axon tracts in vivo, AMIGO might participate in fasciculation of neurites. We addressed this question by a dominant negative approach using the ectodomain of AMIGO as Ig-fusion protein in the culture medium. Hippocampal neurons were plated on poly-L-lysine coated wells to promote neurite outgrowth and fasciculation. Microscopy of the cultures revealed that the growth pattern of neurites was dramatically changed in the presence of the soluble AMIGO. In the control cultures neurites formed fascicles in 4 days, where as in the presence of the soluble AMIGO, the processes were mainly non-fasciculated up to at least 5 days in culture (Fig. 9 A-C).

AMIGO Displays a Homophilic Binding Mechanism

Fasciculation of axons is known to involve homophilic interactions and this might be reason why soluble AMIGO perturbs fasciculation. We therefore tested in a coimmunoprecipitation assay wether AMIGO could bind to itself. To examine AMIGO-AMIGO association, 293 cells were cotransfected with GFP-tagged full length AMIGO (Fig.10 A, lanes 1-4) and V5-tagged full length AMIGO (Fig. 10 A, lane 1) and soluble V5-tagged AMIGO ectodomain (Fig. 10 A, lane 2). Immunoprecipitation of both AMIGO-V5 forms from the cell lysates precipitated AMIGO-GFP (Fig. 10 A, lanes 1 and 2) and correspondingly both the full length and soluble AMIGO-V5 were precipitated with anti-GFP (Fig. 10 A, lanes 1 and 2). No coimmunoprecipitation was observed when V5-tagged AMIGO was not transfected into cells (Fig. 10 A, lane 3). The control protein V5-tagged human RAGE was not coprecipitated with the AMIGO-GFP and vice versa. (Fig. 10 A, lane 3).

As another approach to study homophilic binding of AMIGO, we added AMIGO Ig-fusion protein to protein-A coated beads to get the protein oriented in a manner that occurs at the cell surface. AMIGO caused rapid aggregation of the beads (Fig. 10 B and C), whereas addition of the control protein IgG Fc part into the beads did not induce any aggregation (Fig. 10 B and D).

Coimmunoprecipitation of AMIGO and AMIGO2 with EGFR

The result shows that both AMIGO and AMIGO2 bind the EGFR and only the EC-part is enough for the binding (shown for the AMIGO, Figure 27).

AMIGO inhibits EGFR phosphorylation

When AMIGO and flag-tagged human EGFR are expressed together AMIGO could clearly inhibit the EGFR autophosphorylation induced by EGF ligation when compared to AMIGO2, AMIGO3 and vector control (Figure 29).

Homo- and heterophilic binding of AMIGO, AMIGO2 and AMIGO3

The coimmunoprecipitation results show that AMIGOs could bind each others in heterophilically but they also posses homophilic binding properties (Figure 28).

Discussion

A Novel Family of Transmembrane Proteins with Six LRR Domains and One Ig-like Domain

In this study, we have identified a novel family of transmembrane proteins called AMIGO, AMIGO2 and AMIGO3. These three proteins show clear homology with each other; their length and location of different domains are highly identical (Fig. 2 B). This domain relationship suggests a common evolutionary origin of the AMIGOs.

Based on genomic sequence data these three proteins probably occur in the puffer fish *Fugu rubripes* (data not shown). Interestingly, *Drosophila* has a protein family called kekkon with three members of transmembrane proteins kek1, kek2 (Musacchio and Perrimon, 1996) and kek3 (Ashburner et al., 1999) which show homology in their

extracellular parts with the AMIGOs . The extracellular parts of both the AMIGOs and the kek proteins contain six LRR domains flanked with cysteine-rich LRRNT and LRRCT domains and one immunoglobulin domain close to the transmembrane region. However, the cytoplasmic parts of the AMIGOs and kek proteins do not display homology with each other. The gene expression data of kek1 and kek2 (Musacchio and Perrimon, 1996) reminds the one seen for AMIGO and AMIGO2; they all are expressed in the central nervous system of the adult organism. These domain and expression similarities suggest that the AMIGOs and kek proteins may be derived from a common ancestral gene.

In their extracellular parts the most homologous motifs between the AMIGOs are the LRRs 3-5. The best fit in BLAST searches shows homology with Slit family of extracellular axon-guiding proteins (Whitford et al 2002), and a clear homology is also found with the Nogo-66 receptor where the only recognizable motifs are the LRR domains (Fournier et al. 2001)(Fig 11). The similarity found in the LRRs in AMIGO, Slit1 and Nogo-66 receptor suggests an evolutionary origin of these proteins from a common ancestor. The clear conservation seen at the LRR area between the AMIGOs suggests that this region is important for interactions with extracellular ligand(s) and that they could also share the same binding partner(s).

In the literature there are reports of other transmembrane proteins that contain LRRs and Ig domains in the extracellular part of the proteins: ISLR (Nagasawa et al., 1997): 5 LRRs and 1 Ig domain; Pal (Gomi et al., 2000): 5 LRRs and 1 Ig domain; LIG-1 (Suzuki et al., 1996): 15 LRRs and 3 Ig domains and GAC1 (Almeida et al., 1998): 12 LRRs and 1 Ig domain. Common for all of these proteins and the AMIGOs is the order of how the LRRs and the Ig domain(s) are organized; the LRRs are always more distal to the transmembrane region than the Ig domain(s). Interestingly, BLAST searches by using Ig-domain sequences from AMIGOs give no clear homology with other Ig-domains of the Ig-superfamily proteins but the most closest are the ones found in proteins containing both Ig and LRR domains (data not shown).

Although the cytoplasmic moieties of the AMIGOs do not display any clear homology with previously identified transmembrane proteins, the alignment of the AMIGOs (Fig. 2 A) shows two conserved serine-rich regions; one close to the transmembrane domain and the other at the C-terminus. The C-terminal serine-rich area of AMIGO and AMIGO2 have

a consensus sequence for Casein kinase II (CK2) serine/threonine kinase (Allende et al. 1995) which is ubiquitously expressed in brain but AMIGO3, which is not expressed in the brain, does not have this consensus sequence. Recently Watts et al. (1999) showed that the transmembrane form of TNF-α has a consensus sequence SXXS which is a substrate for Casein kinase I (CK1) dependent phosphorylation. Interestingly, all three AMIGOs have four possible CK1 phoshorylation sites in these two conserved serine rich areas. Future work will reveal whether these conserved serine residues have important functions in signalling events of the AMIGOs.

There are increasingly reports in the literature and the data banks on mammalian transmembrane proteins with both LRR and Ig domains but unfortunately at present almost all data only comprise the cloning and tissue expression of these proteins. Our data here gives a functional insight into these twin motif transmembrane proteins, belonging to both the LRR and Ig superfamily, in a form of more detailed characterization of AMIGO.

AMIGO, A Novel Transmembrane Protein in Neuronal Processes with Homophilic Binding Mechanism

Based on RT-PCR experiments, in situ hybridization and immunohistochemistry, AMIGO is an essentially nervous system specific protein. Interestingly, AMIGO expression is upregulated at two clearly distinct stages during brain development: the first peak is found perinatally, and the second upregulation occurs during or slightly before the upregulation of the oligodendrocyte-specific marker α-CNPase.

The first expression peak of AMIGO would be compatible with a role in growth of axonal connections. The expression of AMIGO in developing axon tracts both in vivo and in vitro and our neurite outgrowth experiments support this role. One cellular mechanism in the growth of axonal connections is fasciculation: axons grow along each other by using pioneer axons as the substratum for the growth cones of the later ones. Interestingly, a dominant negative approach using AMIGO ectodomain in the culture medium clearly suggests a role for AMIGO in fasciculation. Further, AMIGO displays a homophilic binding mechanism that would explain its role in fasciculation. Homophilic adhesion molecules belonging to both the Ig-superfamily and to the cadherin family have been shown to mediate neurite outgrowth and fasciculation during the nervous system development (for reviews, see Kamiguchi and Lemmon 1997; Martinek and Gaul 1997). It

is also noteworthy that the LRR sequences of the AMIGOs display homology with the slit proteins and with the Nogo receptor (Fig. 11) that have been implicated in axon growth, regeneration and guidance.

The second upregulation of the AMIGO expression suggests a role in myelination. It seems reasonable that AMIGO would mediate cell-to-cell interactions also at this stage of development. However, further studies are clearly warranted to understand the role of AMIGO in myelinating axon tracts, like in the interactions of axons with oligodendrocytes and Schwann cells. Further, AMIGO expression remains high until adulthood. This suggests that AMIGO plays a role in regeneration and plasticity of the adult fiber tracts, the mechanisms of which commonly recapitulate mechanisms of fiber tract development.

To get further insight into the functional roles of AMIGO during development and adulthood, we have recently targeted the gene in ES cells and are currently producing AMIGO null mice (Kuja-Panula and Rauvala, unpublished results). In addition to the in vivo approaches using gene targeting, it will be important to understand what molecular domains mediate homophilic binding and whether the intracellular domain of AMIGO has signalling properties. Furthermore, future studies will reveal whether the members of the AMIGO family mediate analogous cell-to-cell interactions in non-neuronal tissues characterized in the present paper for AMIGO in axonal tracts.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

The publications and other materials used herein to illuminate the background of the invention, and in particular, to provide additional details with respect to its practice, are incorporated herein by reference.

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CLAIMS

1. A purified and isolated AMIGO nucleic acid comprising a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, 4 or 6.

2. A purified and isolated nucleic acid comprising a nucleotide sequence shown in SEQ ID NO:1, 3 or 5.

3. A purified and isolated nucleic acid comprising a recombinant nucleotide sequence comprising a nucleotide sequence shown in SEQ ID NO:1, 3 or 5 or a homolog or fragment thereof.

4. An expression construct comprising the nucleic acid according to claim 2 operatively linked to an expression control sequence, said expression construct capable of encoding an AMIGO polypeptide or variants thereof.

5. A host cell transformed or transfected with the expression construct of claim 4.

6. A host cell transformed or transfected with a polynucleotide wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO:1, 3 or 5, under the following hybridization conditions:

(a) hybridization at 42 °C for 20 hours in a solution containing 50% formamide, 5 X SSPE, 5 X Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65 °C with a wash solution containing 1xSSC, and 0.1% SDS.

7. An isolated and purified AMIGO polypeptide comprising the amino acid sequence of SEQ ID NO:2, 4 or 6.

8. Method of producing an AMIGO polypeptide according to claim 7, said method comprising the steps of:
culturing a host cell of claim 5 comprising a polynucleotide encoding said polypeptide operably associated with a promoter sequence such that the nucleic acid sequence encoding said polypeptide is expressed; and
isolating said polypeptide from said host cell or from a growth medium in which said host cell is cultured.

9. Method of producing antibodies comprising:
- immunising a mammal with the isolated and purified AMIGO protein of claim 7 or an antigenic fragment thereof.

10. Use of the isolated and purified AMIGO protein of claim 7 or an antigenic fragment thereof as an antigen.

11. An antibody produced by the method of claim 9.

12. The antibody of claim 11 which is labeled with a detectable label.

13. A kit of reagents for use in detecting the presence of AMIGO or allelic variant thereof in a biological sample, comprising
- a container; and in said container:
- a compound, preferably labeled, capable of detecting AMIGO or allelic variants thereof.

14. The kit according to claim 13, wherein said compound is a primer or probe.

15. The kit according to claim 13, wherein said compound is an antibody as defined in claim 11.

16. The kit according to any one claims 13-14 for assessing the predisposition of an individual to a condition mediated by variation or dysfunction of AMIGO.

17. The kit according to claim 16 further comprising instructions for using the kit.

18. A transgenic non-human animal containing a human or murine AMIGO gene as a transgene.

19. A transgenic non-human animal containing a transgene or insertion disrupting expression of an AMIGO gene or a homolog thereof.

20. A pharmaceutical compound comprising AMIGO nucleic acid molecule, AMIGO protein, AMIGO peptide fragment, AMIGO fusion protein, AMIGO agonists, AMIGO antagonists or anti-AMIGO antibody.

21. Method for treatment of a condition dependent on AMIGO wherein a pharmaceutically effective amount of the compound of claim 20 is administered to a patient in need of such treatment.

22. Method for affinity purification of ligand that binds to the AMIGO comprising the following steps: a) contacting a source of AMIGO receptor with an immobilized AMIGO under conditions whereby the AMIGO receptor to be purified is selectively adsorbed onto the immobilized AMIGO; (b) washing the immobilized AMIGO and its support to remove non-adsorbed material; and (c) eluting the AMIGO receptor molecules from the immobilized AMIGO to which they are adsorbed with an elution buffer.

23. A method for identifying a modulator of binding between an AMIGO receptor and an AMIGO receptor, comprising steps of:

(a) contacting an AMIGO receptor composition with an AMIGO composition in the presence and in the absence of a putative modulator compound;

(b) detecting binding between AMIGO receptor and the AMIGO receptor in the presence and absence of the putative modulator; and

(c) identifying a modulator compound in view of decreased or increased binding between the AMIGO receptor and the AMIGO receptor in the presence of the putative modulator, as compared to binding in the absence of the putative modulator.

24. A method according to claim 23, further comprising a step of:

(d) making a modulator composition by formulating a modulator identified according to step (c) in a pharmaceutically acceptable carrier.

25. A method according to claim 24, further comprising a step of:

(e) administering the modulator composition to an animal that comprises cells that express the AMIGO receptor, and determining physiological effects of the modulator composition in the animal.

26. A method according to any one of claims 23-25, wherein the AMIGO receptor composition comprises a member selected from the group consisting of:

(a) a purified polypeptide comprising a AMIGO receptor extracellular domain fragment that binds the AMIGO;

(b) a phospholipid membrane containing AMIGO receptor polypeptides; and

(c) a cell recombinantly modified to express increased amounts of an AMIGO receptor on its surface.

27. A method according to any one of claims 23-25, wherein the AMIGO receptor composition comprises an AMIGO receptor extracellular domain fragment bound to a solid support.

28. A method according to any one of claims 23-25, wherein the AMIGO receptor composition comprises an AMIGO receptor extracellular domain fragment fused to an immunoglobulin Fc fragment.

29. A method according to any one of claims 23-25, wherein the AMIGO receptor is selected from the group consisting of a mammalian AMIGO, AMIGO2, and AMIGO3.

30. A method according to any one of claims 23-29, wherein the AMIGO receptor is human.

31. A method according to any one of claims 23-30, wherein the AMIGO composition comprises a member selected from the group consisting of:

(a) a purified polypeptide comprising an AMIGO fragment that binds the AMIGO receptor;

(b) a phospholipid membrane containing AMIGO polypeptides; and

(c) a cell recombinantly modified to express increased amounts of an AMIGO on its surface.

32. A method according to any one of 23-30, wherein the AMIGO composition comprises an AMIGO extracellular domain fragment bound to a solid support.

33. A method according to any one of claims 23-30, wherein the AMIGO composition comprises an AMIGO extracellular domain fragment fused to an immunoglobulin Fc fragment.

34. A method according to any one of claims 23-33, wherein the AMIGO is human.

35. A method according to any one of claims 23-25, wherein the AMIGO receptor composition comprises a cell recombinantly modified to express increased amounts of an AMIGO receptor on its surface, and wherein the detecting step comprises measuring an AMIGO binding-induced physiological change in the cell.

36. A method according to any one of claims 23-25, wherein the AMIGO composition comprises a cell recombinantly modified to express increased amounts of an AMIGO on its surface, and wherein the detecting step comprises measuring an AMIGO binding-induced physiological change in the cell.

37. A method for screening for selectivity of a modulator of binding between an AMIGO and an EGFR, comprising steps of:

a) contacting an AMIGO receptor composition with an EGFR composition in the presence and in the absence of a compound that modulates binding between the AMIGO receptor and EGFR receptor; and

b) detecting binding between the AMIGO receptor composition and the EGFR receptor composition in the presence and absence of the modulator compound,

c) identifying the selectivity of the modulator compound in view of decreased or increased binding between the AMIGO receptor and the EGFR receptor in the presence as compared to the absence of the modulator, wherein increased selectivity of the modulator for modulating AMIGO EGFR binding correlates with decreased differences in AMIGO-EGFR binding.

38. A method of modulating growth, migration, axonal growth, myelination, fasciculation or proliferation of cells in a mammalian organism, comprising a step of:

(a) identifying a mammalian organism having cells that express a AMIGO receptor and/or EGFR; and

(b) administering to said mammalian organism a composition, said composition comprising an agent selected from the group consisting of:

(i) a polypeptide comprising an AMIGO receptor that binds to the AMIGO receptor and/or EGFR, or a nucleic acid encoding said polypeptide;

(ii) a polypeptide comprising a fragment of the AMIGO, wherein the polypeptide and fragment retain AMIGO binding characteristics of the AMIGO, or a nucleic acid encoding said polypeptide;

(iii) an antibody that specifically binds the polypeptide of (i) or (ii) in a manner that inhibits the polypeptide from binding the AMIGO receptor and/or EGFR, or a fragment of the antibody that specifically binds the polypeptide of (i) or (ii);

(iv) a polypeptide comprising an antigen-binding fragment of (iii) and that inhibits the polypeptide of (i) or (ii) from binding the AMIGO receptor and/or EGFR;

(v) a molecule that selectively inhibits AMIGO binding to the AMIGO receptor without inhibiting AMIGO binding to the EGFR receptor; and

(vi) a molecule selectively binding to the AMIGO receptor and the EGFR receptor;

wherein the composition is administered in an amount effective to modulate growth, migration, or proliferation of cells that express AMIGO in the mammalian organism.

39. A method according to claim 38, wherein the mammalian organism is human.

40. A method according to claim 38 or 39, wherein the cells comprise neuronal cells.

41. A method according to any one of claims 38-40, wherein the organism has a disease characterized by aberrant growth, migration, or proliferation of neuronal cells/neuronal extensions.

42. A method according to to any one of claims 38-41, wherein the conditions comprises a neuronal trauma.

43. A method according to claim 38, further comprising administering a second agent to the patient for modulating neuronal growth, migration, regeneration or proliferation, said second agent selected from the group consisting of: an antibody that specifically binds with any of the foregoing polypeptides, an antibody that specifically binds with a receptor for any of the foregoing polypeptides, or a polypeptide comprising an antigen binding fragment of such antibodies.

44. A method according to claim 38, wherein the AMIGO extracellular fragment is conjugated with Fc domain.

45. A method according to claim 44, wherein rat AMIGO Fc fusion protein sequences have been replaced essentially with the human AMIGO and Fc sequences

46. A polypeptide according to claims 38-45, for use in the manufacture of a medicament for the treatment of diseases characterized by aberrant growth, migration, regeneration or proliferation of cells that express an AMIGO receptor.

47. Method according to claims 38-45 wherein neuronal cells are selected from the group consisting of: hippocampal cells, cerebral cells, cerebellar cells, neuronal trauma cells, glial scar cells, spinal cord cells, optic nerve cells, retina cells, kidney cells, and cells acting during fasciculation, guidance, growth, or myelination.

48. A method of modulating cancer, tumour growth or metastasis in a mammalian organism, comprising a step of:

(a) identifying a mammalian organism having cells that express an AMIGO receptor and/or EGFR; and

(b) administering to said mammalian organism a composition, said composition comprising an agent selected from the group consisting of:

(i) a polypeptide comprising an AMIGO receptor that binds to the AMIGO receptor and/or EGFR, or a nucleic acid encoding said polypeptide;

(ii) a polypeptide comprising a fragment of the AMIGO, wherein the polypeptide and fragment retain AMIGO binding characteristics of the AMIGO, or a nucleic acid encoding said polypeptide;

(iii) an antibody that specifically binds the polypeptide of (i) or (ii) in a manner that inhibits the polypeptide from binding the AMIGO receptor and/or EGFR, or a fragment of the antibody that specifically binds the polypeptide of (i) or (ii);

(iv) a polypeptide comprising an antigen-binding fragment the (iii) and that inhibits the polypeptide of (i) or (ii) from binding the AMIGO receptor and/or EGFR;
(v) a molecule that selectively inhibits AMIGO binding to the AMIGO receptor without inhibiting AMIGO binding to the EGFR receptor; and
(vi) a molecule selectively binding to the AMIGO receptor and the EGFR receptor;

wherein the composition is administered in an amount effective to modulate cancer growth or metastasis of cells that express AMIGO in the mammalian organism.

49. A method according to claim 48, wherein the mammalian organism is human.

50. A method according to claim 48 or 49, wherein the cells comprise glioma, glioblastoma, astrocytoma, anaplastic astrocytoma, ependymomas, oligodendrogliomas, medulloblastomas, meningiomas, schwannomas, craniopharyngiomas, germ cell tumors, pineoblastoma, pineocytoma, germinoma cells, lung carcinoma, breast carcinoma, ovarian carcinoma, colorectal carcinoma, bladder carcinoma, pancreatic carcinoma, squamous cell carcinoma, or renal carcinoma cells.

51. A method according to any one of claims 48-50, wherein the organism has a disease characterized by cancer or metastasis.

52. A method according to claim 51, wherein the condition comprises a brain tumor.

53. A method according to claim 48, further comprising administering a second agent to the patient for modulating cancer growth or metastatic growth of cancer, said second agent selected from the group consisting of: an antibody that specifically binds with any of the foregoing polypeptides, an antibody that specifically binds with a receptor for any of the foregoing polypeptides, or a polypeptide comprising an antigen binding fragment of such antibodies.

54. A method according to claim 48, wherein the AMIGO extracellular fragment is conjugated with Fc domain.

55. A method according to claim 48, wherein rat AMIGO Fc fusion protein sequences have been replaced essentially with the human AMIGO and Fc sequences

56. Method for treatment of cancer or metastatic growth of cancer cells selected from the group consisting of: glioma, glioblastoma, astrocytoma, anaplastic astrocytoma, ependymomas, oligodendrogliomas, medulloblastomas, meningiomas, schwannomas, craniopharyngiomas, germ cell tumors of germinoma cells, lung carcinoma, breast carcinoma, ovarian carcinoma, colorectal carcinoma, bladder carcinoma, pancreatic carcinoma, squamous cell carcinoma, and renal carcinoma, comprising a step of administering to a subject in need of such treatment the compound as claimed in claim 20.

57. Method for treatment of neuronal cells selected from the group consisting of: hippocampal cells, cerebral cells, cerebellar cells, neuronal trauma cells, glial scar cells, spinal cord cells, optic nerve cells, retina cells, kidney cells, and cells acting during fasciculation, guidance, growth, or myelination, comprising a step of administering to a subject in need of such treatment the compound as claimed in claim 20.

58. A polypeptide or a nucleic acid encoding said polypeptide, said polypeptide comprising a fragment of an AMIGO that binds to an AMIGO receptor, for use in the manufacture of a medicament for the treatment of diseases characterized by aberrant growth, migration, regeneration or proliferation of cells that express an AMIGO receptor.

59. A method of modulating the phosphorylation of a human epidermal growth factor receptor in cells or tissues comprising contacting said cells or tissues with the AMIGO compounds.

60. The method of claim 59, wherein said AMIGO compounds comprises AMIGO peptides encoded a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5.

61. The method of claim 59, wherein said AMIGO compounds comprise an anti-AMIGO antibody.

Figure 1A and 1B

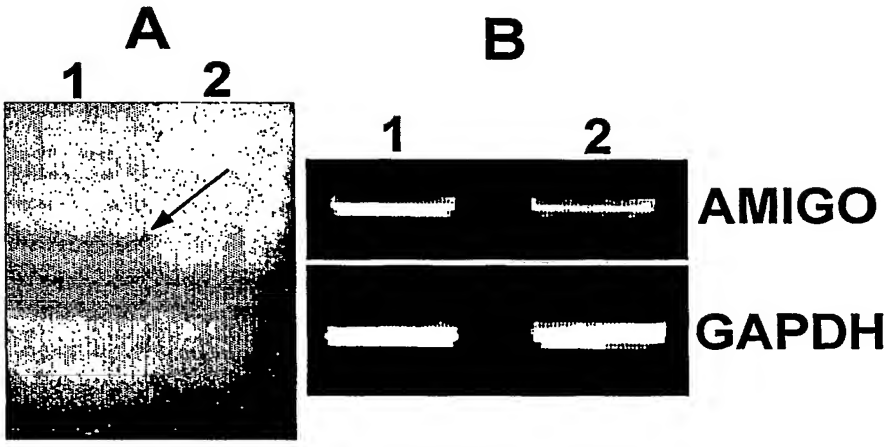


Figure 2A and 2B

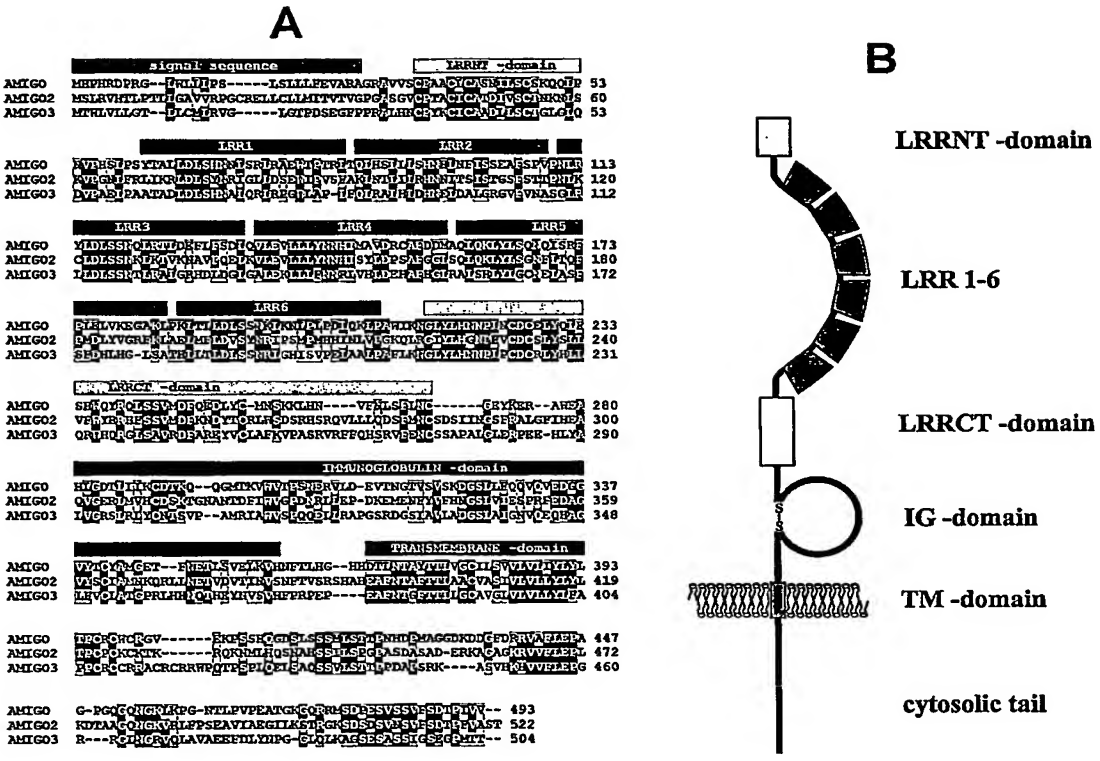


Figure 3

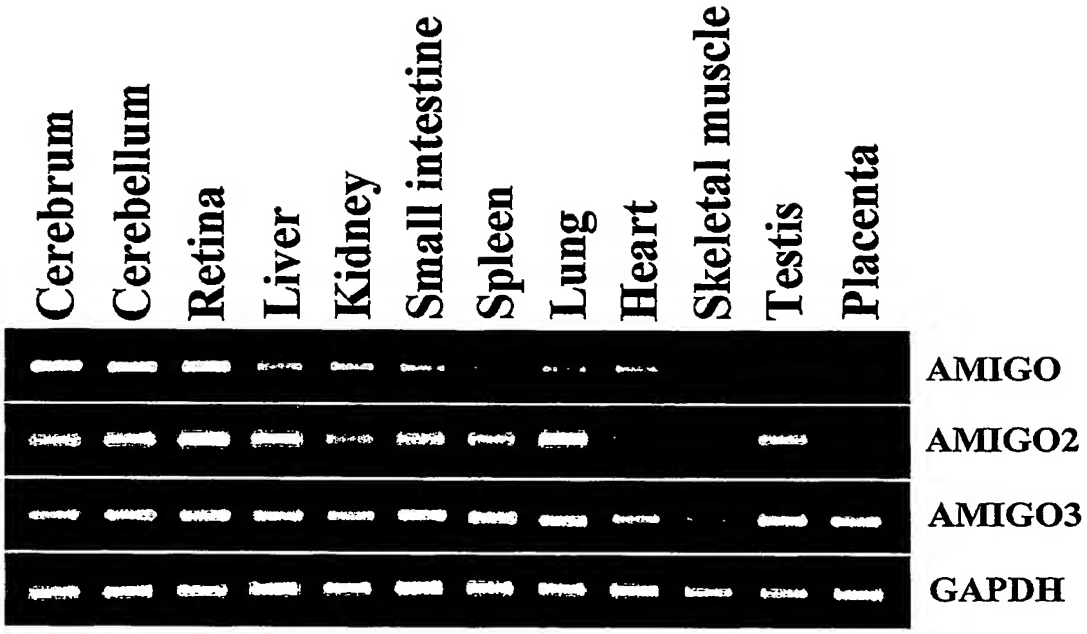

Cerebrum
Cerebellum
Retina
Liver
Kidney
Small intestine
Spleen
Lung
Heart
Skeletal muscle
Testis
Placenta
AMIGO
AMIGO2
AMIGO3
GAPDH

Figure 4A, 4B and 4C

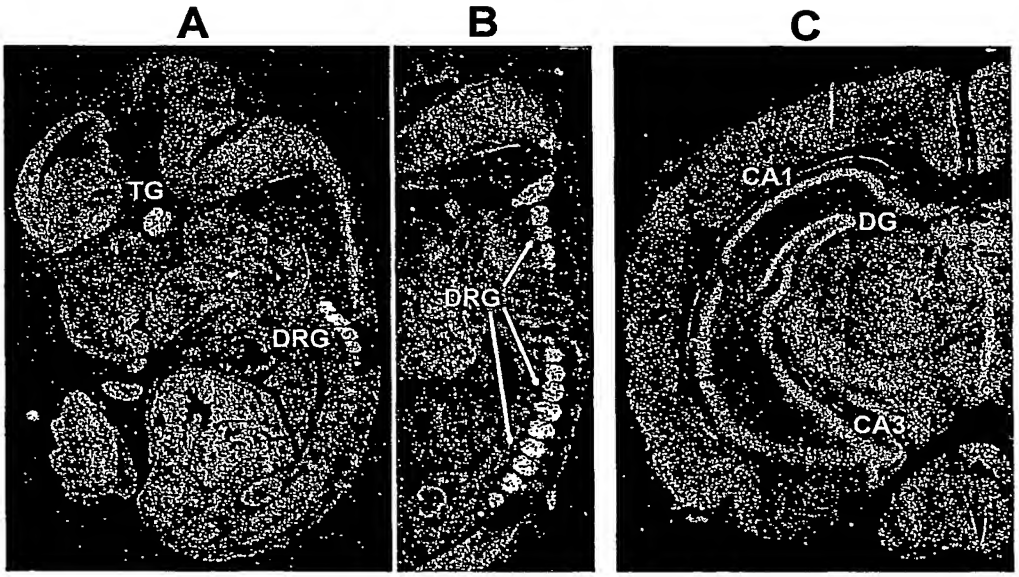


Figure 5A and 5B

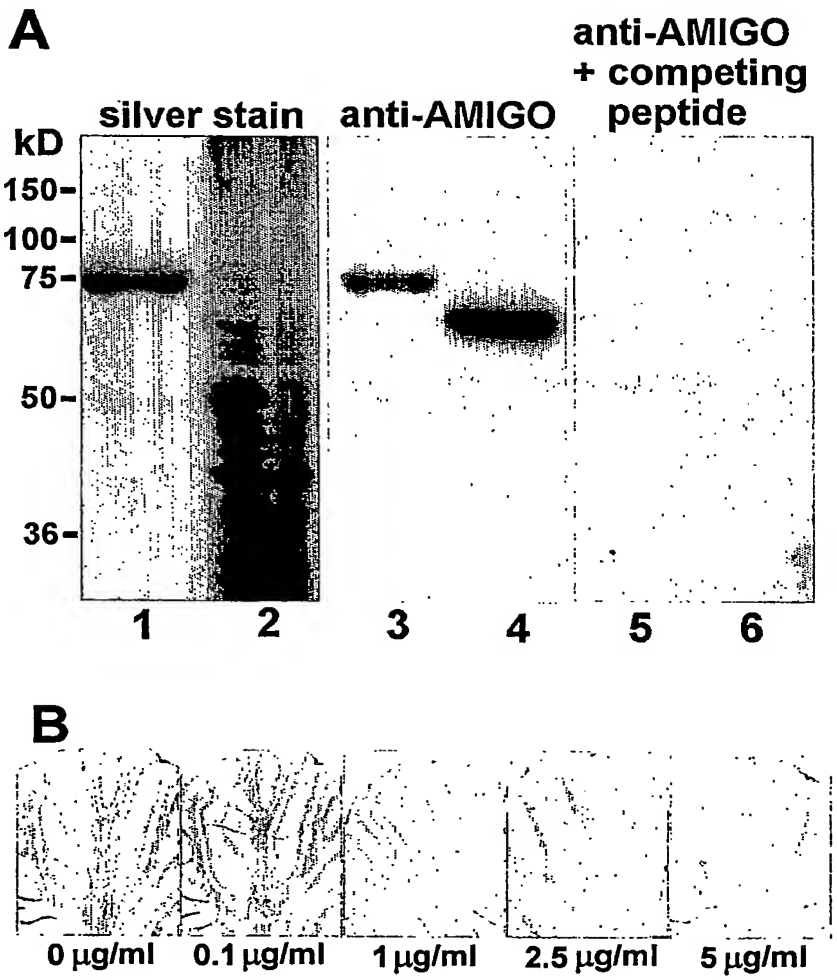


Figure 6

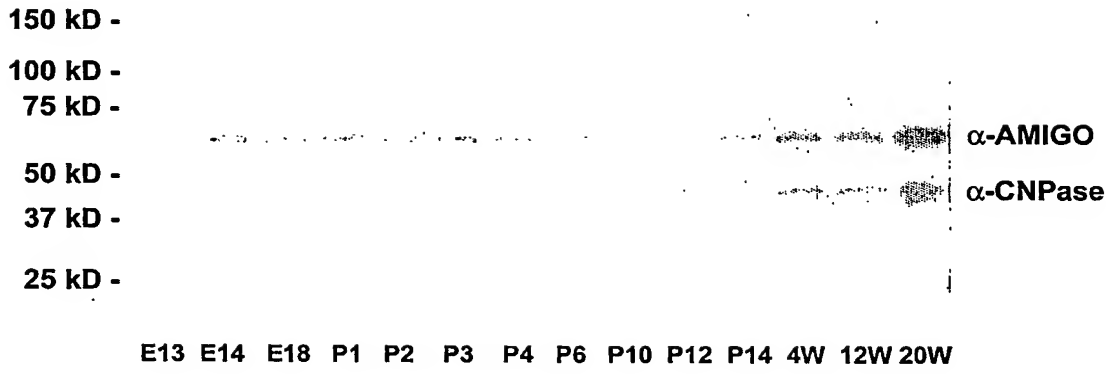

150 kD -
100 kD -
75 kD -
50 kD -
37 kD -
25 kD -
α-AMIGO
α-CNPase
E13 E14 E18 P1 P2 P3 P4 P6 P10 P12 P14 4W 12W 20W

Figure 7A, 7B, 7C, 7D, 7E and 7F

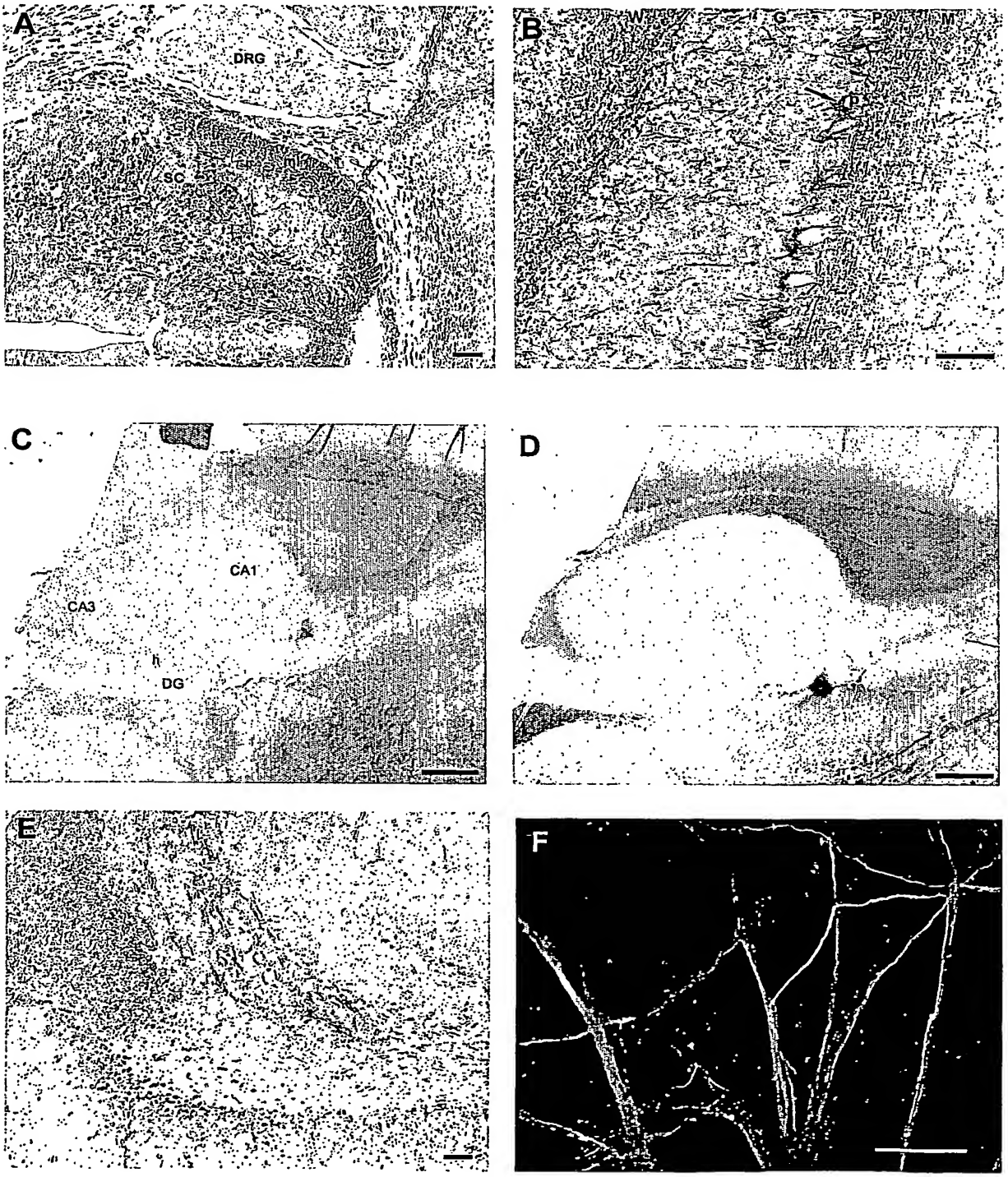


Figure 8A, 8B, 8C and 8D

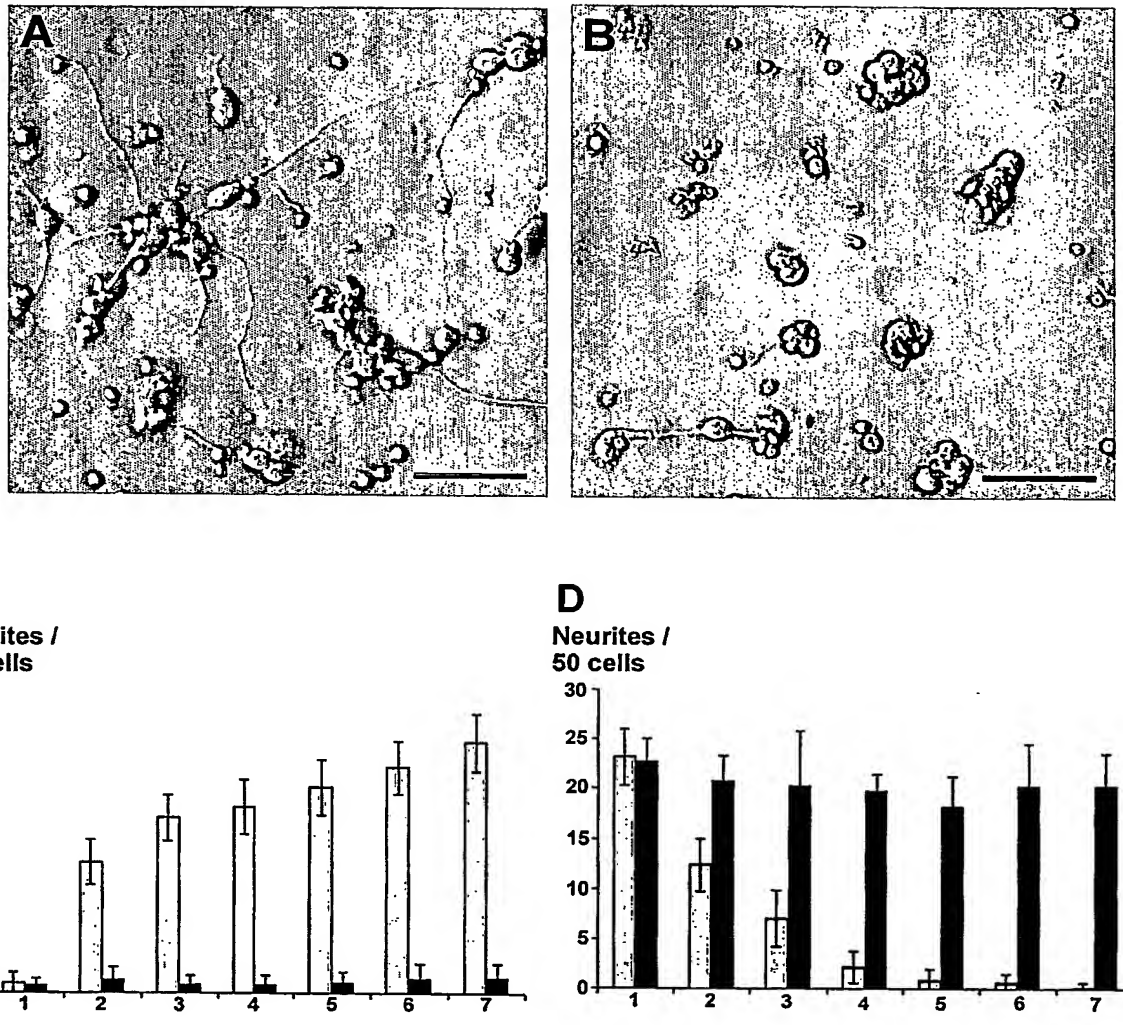
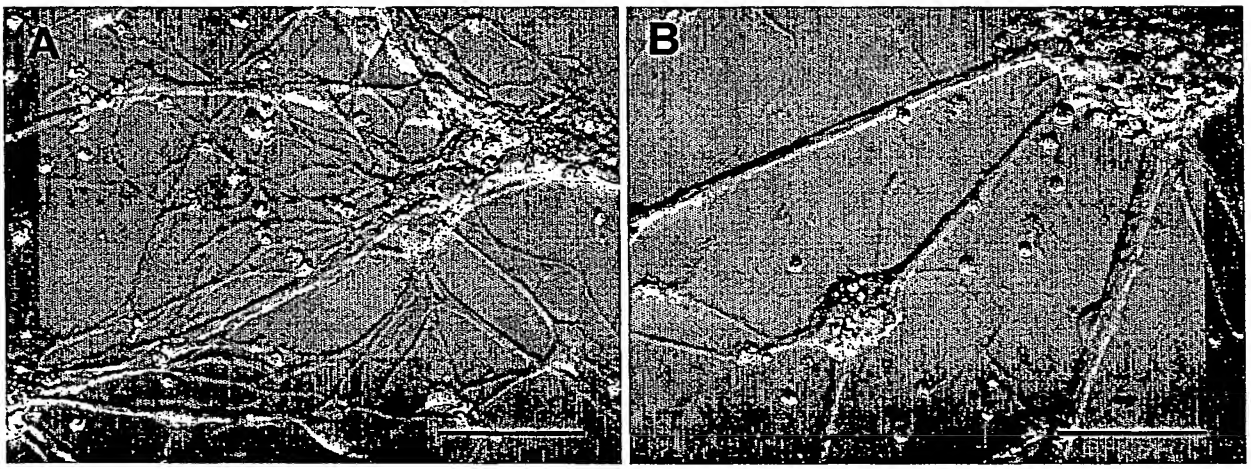


Figure 9A, 9B and 9C



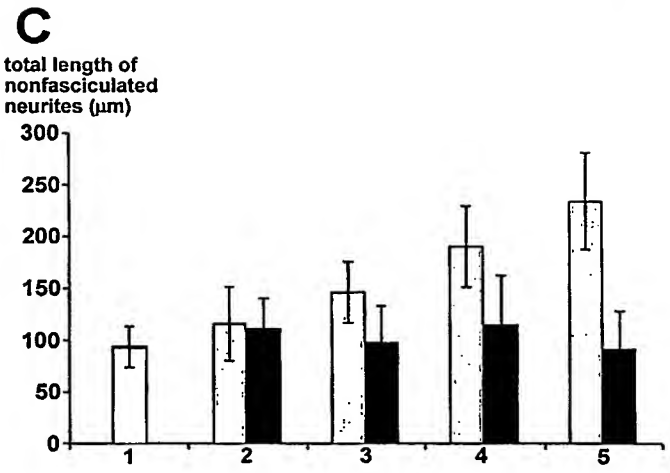


Figure 10A, 10B, 10C and 10D

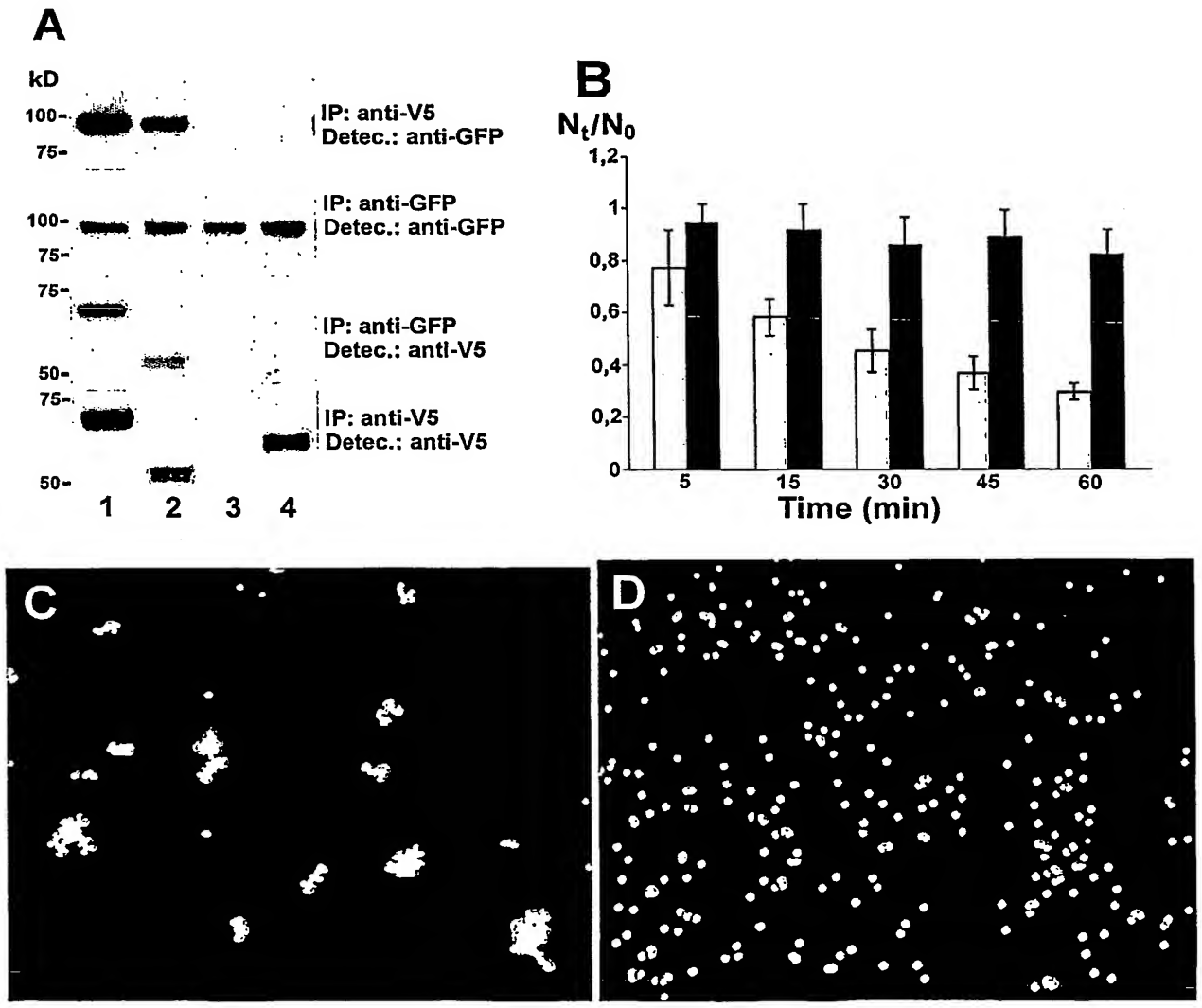


Figure 11

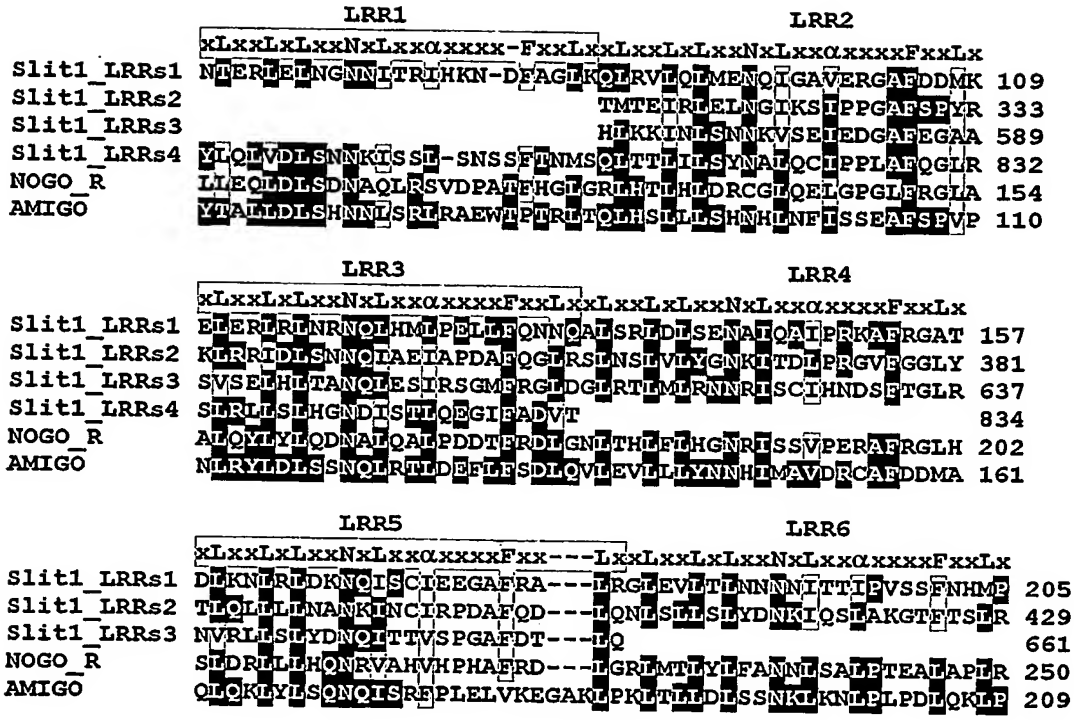
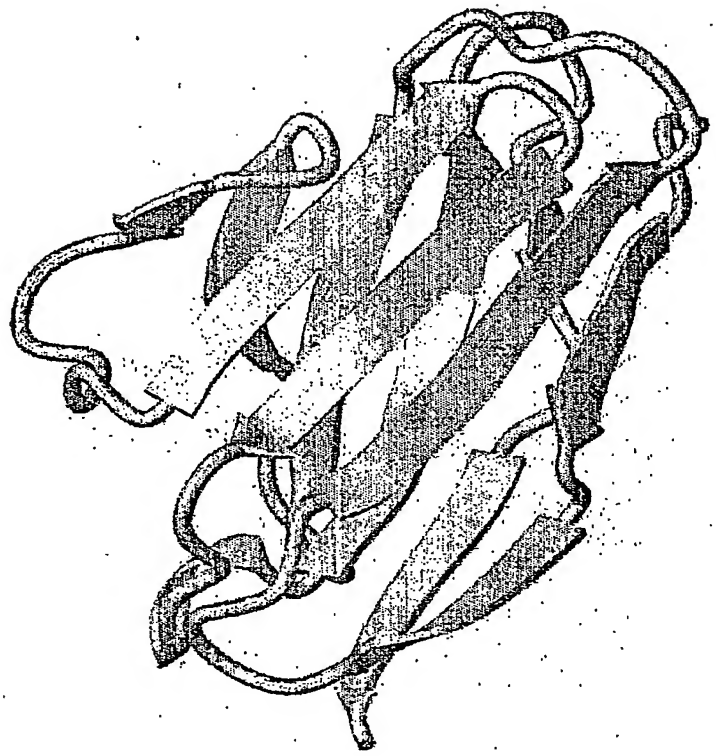
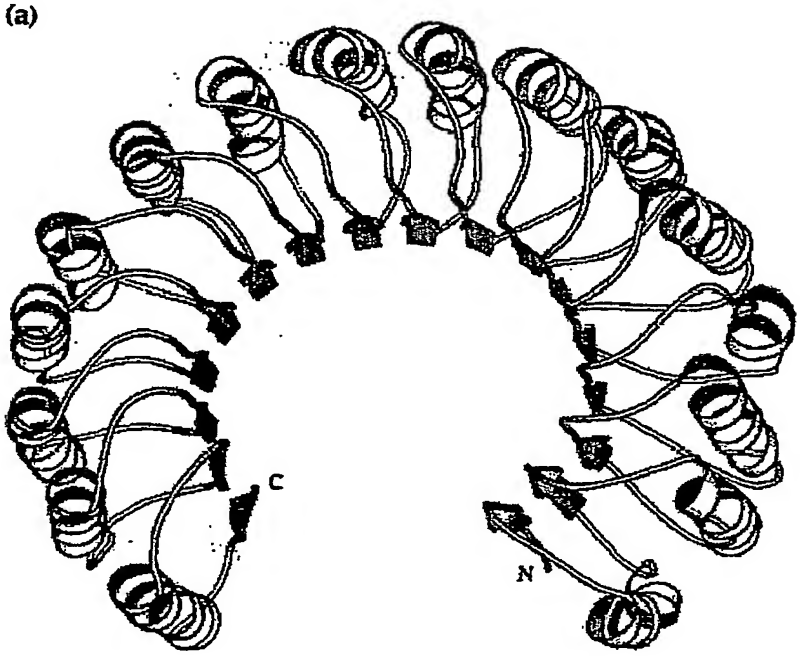

LRR1
LRR2
xLxxLxLxxNxLxxαxxxx-FxxLxxLxxLxLxxNxLxxαxxxxFxxLx
Slit1_LRRs1 NTERIELNGNNITRIHKN-DFAGLKQLRVLQLMENQIGAVERGAFDDMK 109
Slit1_LRRs2 TMTEIRLELNGIKSIPPGAFSPYR 333
Slit1_LRRs3 HLKKINLSNNKVSEIEDGAFEGAA 589
Slit1_LRRs4 YLQLVDLSNNKISSL-SNSSFTNMSQLTTLILSYNALQCIPPLAFQGLR 832
NOGO_R LLEQLDLSDNAQLRSVDPATFHGLGRLHTLHLDRCGLQELGPGLFRGLA 154
AMIGO YTALLDLSHNNLSRLRAEWTPTRLTQLHSLLLSHNHLNFISSEAFSPVP 110
LRR3
LRR4
xLxxLxLxxNxLxxαxxxxFxxLxxLxxLxLxxNxLxxαxxxxFxxLx
Slit1_LRRs1 ELERLRLNRNQIHMLPELLEQNNQALSRLDLSENAIQAIPRKAFRGAT 157
Slit1_LRRs2 KLRRIDLSNNQIAEIAPDAFQGLRSLNSLVLYGNKITDLPRGVFGGLY 381
Slit1_LRRs3 SVSELHLTANQLESIRSGMFRGLDGLRTLMLRNNRISCIHNDSFTGLR 637
Slit1_LRRs4 SLRLLSLHGNDISTLQEGIFADVT 834
NOGO_R ALQYLYLQDNALQALPDDTFRDLGNLTHLFLHGNRISSVPERAFRGLH 202
AMIGO NLRYLDLSSNQLRTLDEFLFSDLQVLEVLLLYNNHIMAVDRCAFDDMA 161
LRR5
LRR6
xLxxLxLxxNxLxxαxxxxFxx---LxxLxxLxLxxNxLxxαxxxxFxxLx
Slit1_LRRs1 DLKNLRLDKNQISCIEEGAFRA---LRGLEVLTLNNNNITTIPVSSFNHMP 205
Slit1_LRRs2 TLQLLLLNANKINCIRPDAFQD---LQNLSLLSLYDNKIQSLAKGTFTSLR 429
Slit1_LRRs3 NVRLLSLYDNQITTVSPGAFDT---LQ 661
NOGO_R SLDRLLLHQNRVAHVHPHAFRD---LGRLMTLYLFANNLSALPTEALAPLR 250
AMIGO QLQKLYLSQNQISRFPLELVKEGAKLPKLTLLDLSSNKLKNLPLPDLQKLP 209

Figure 12



(a)



(b)

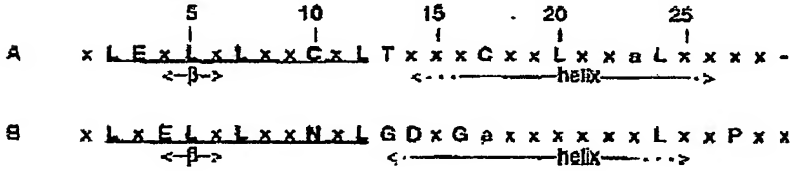


Figure 13A and 13B

Figure 14

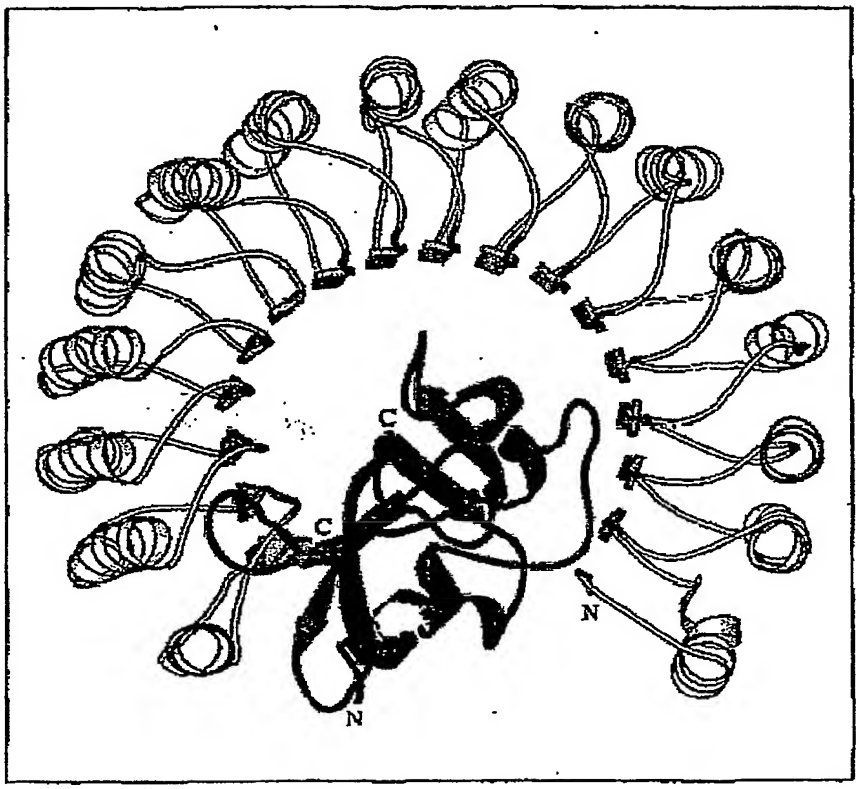


Figure 15

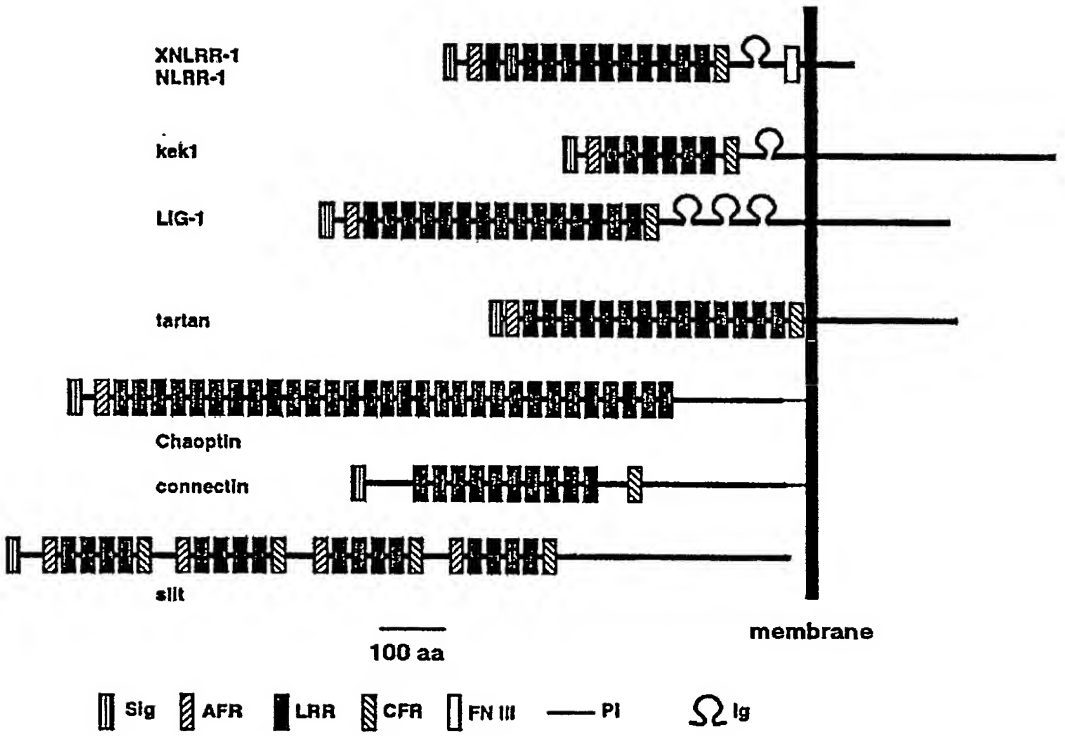

XNLRR-1
NLRR-1
kek1
LIG-1
tartan
Chaoptin
connectin
slit
membrane
100 aa
Sig
AFR
LRR
CFR
FN III
PI
Ig

Figure 16

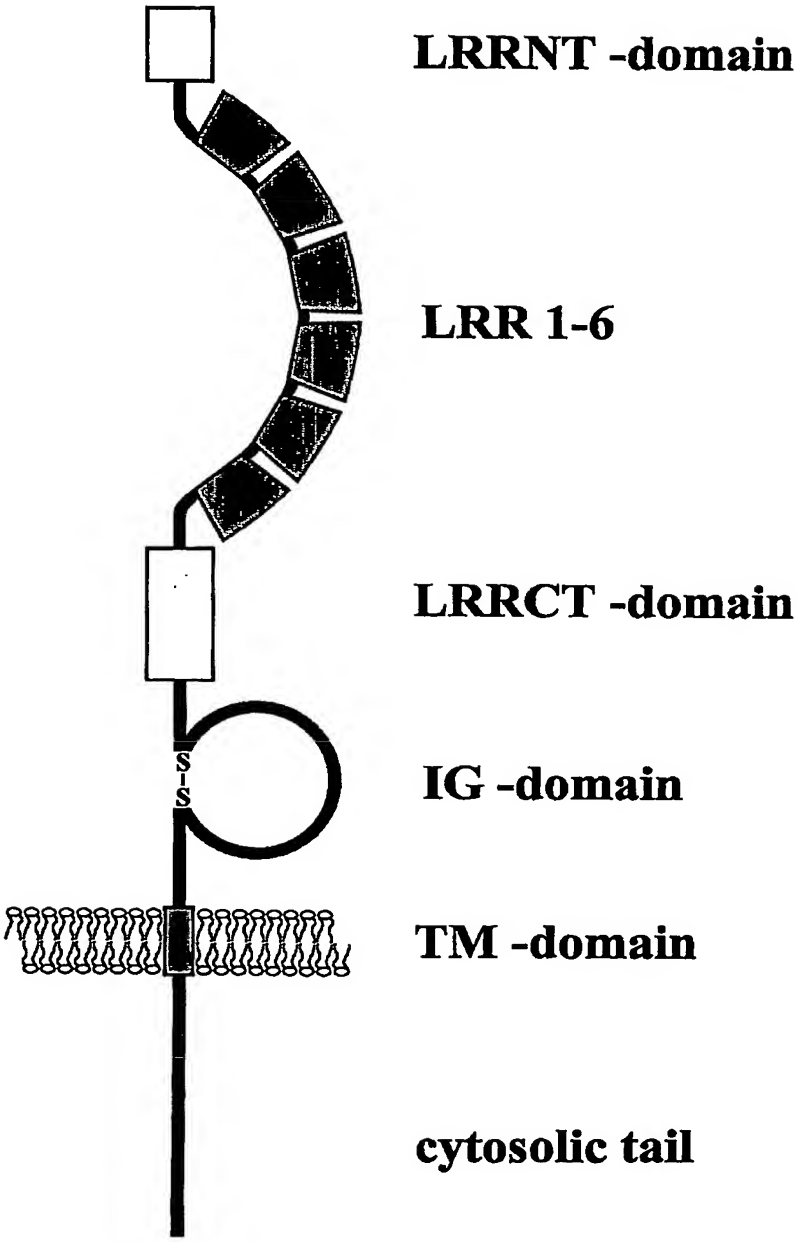

LRRNT -domain
LRR 1-6
LRRCT -domain
S
S
IG -domain
TM -domain
cytosolic tail

Figure 17

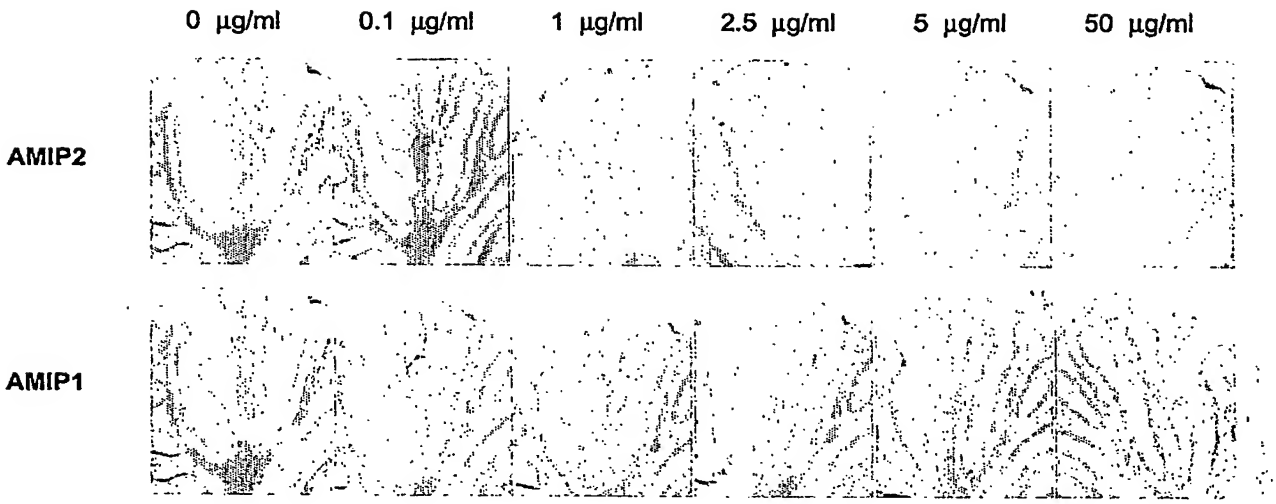


Figure 18

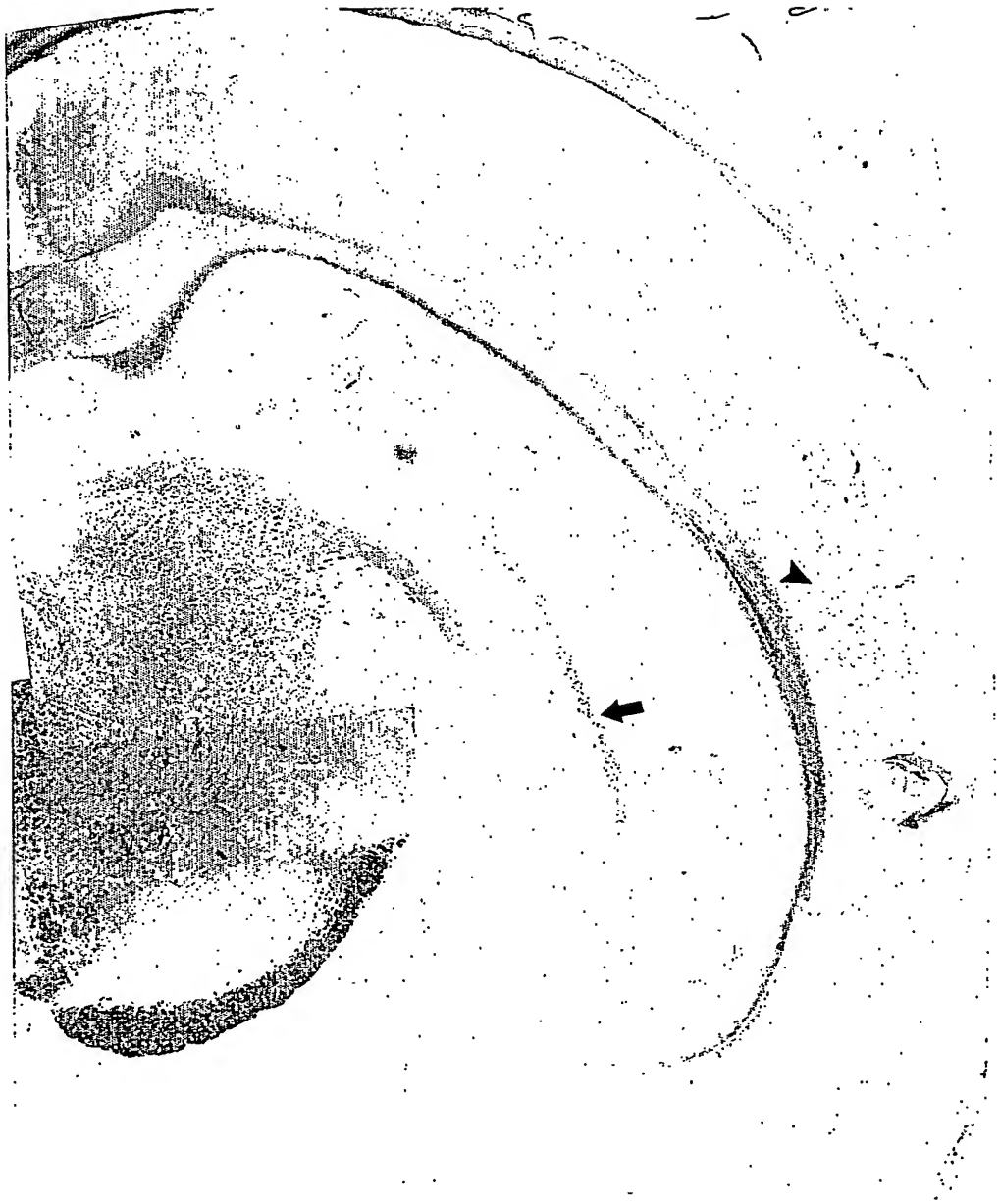


Figure 19A and 19B

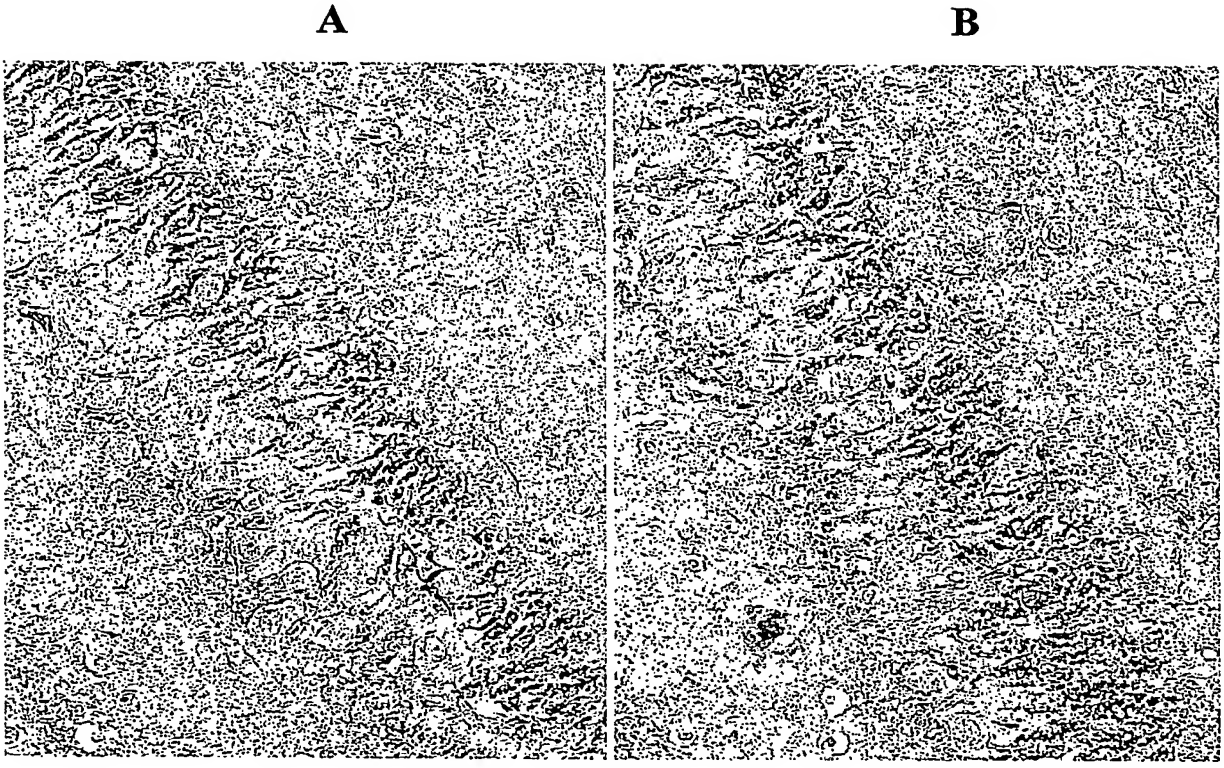


Figure 20A, 20B, 20C and 20D

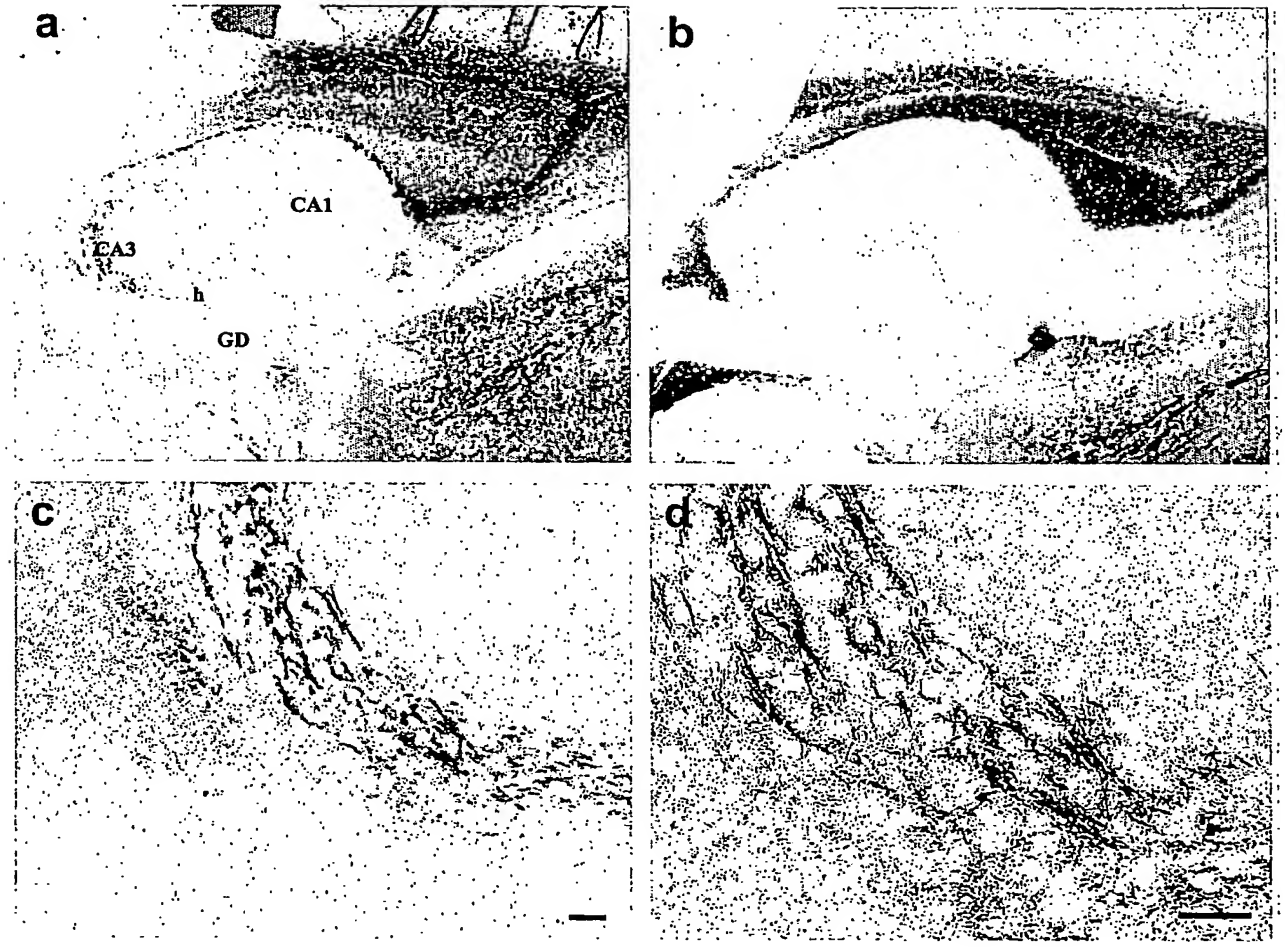


Figure 21A and 21B

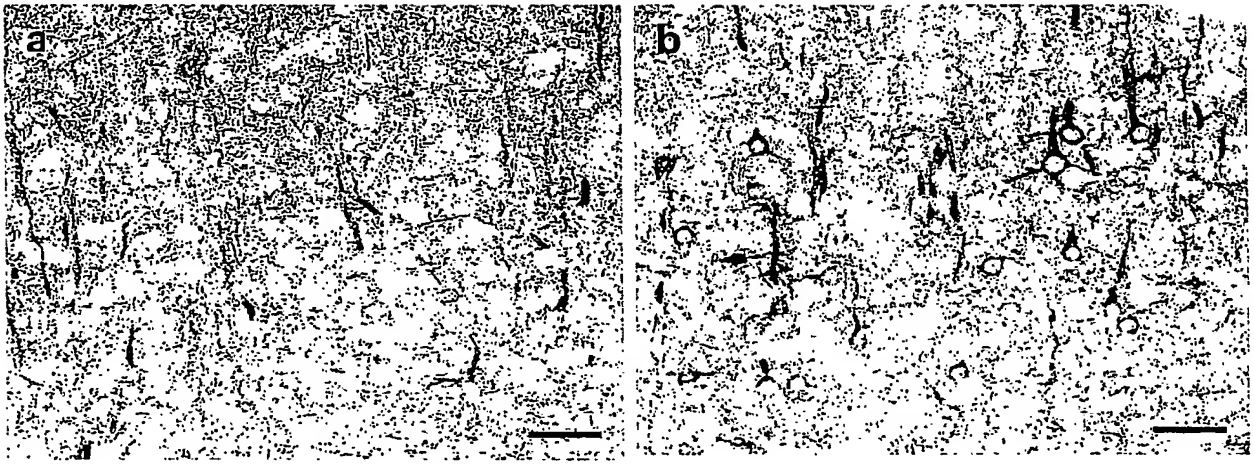


Figure 22A and 22B

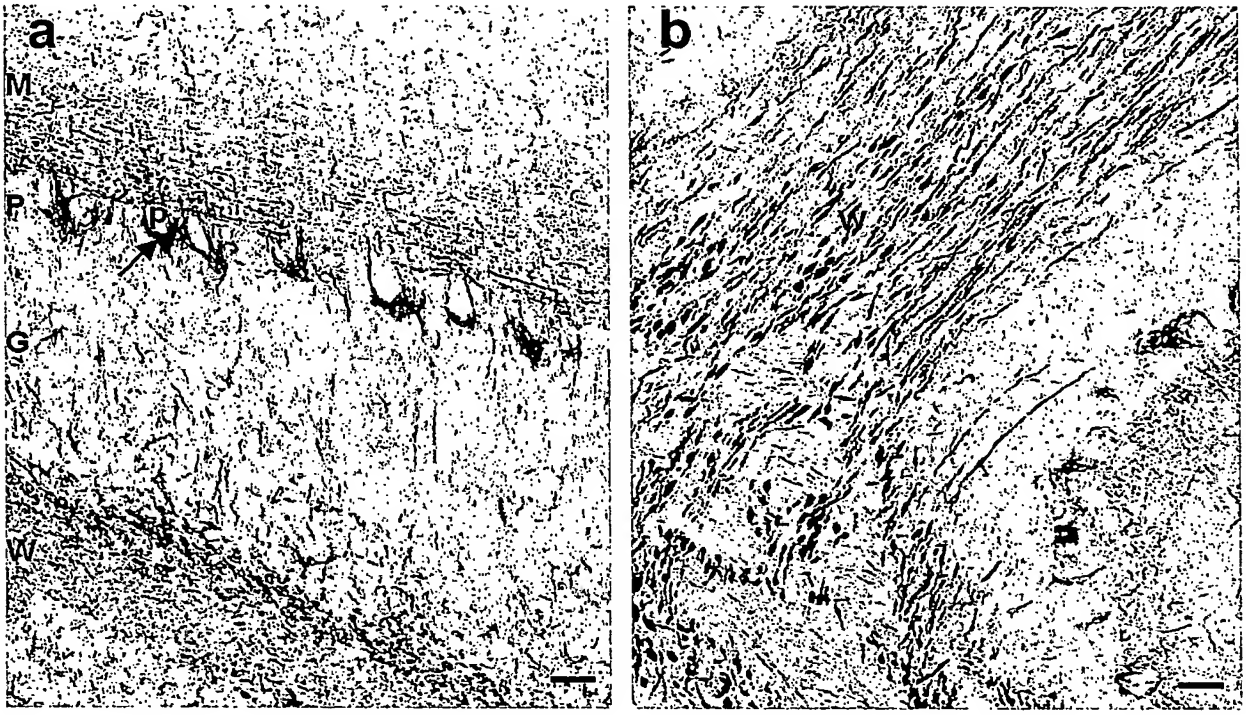


Figure 23A, 23B and 23C

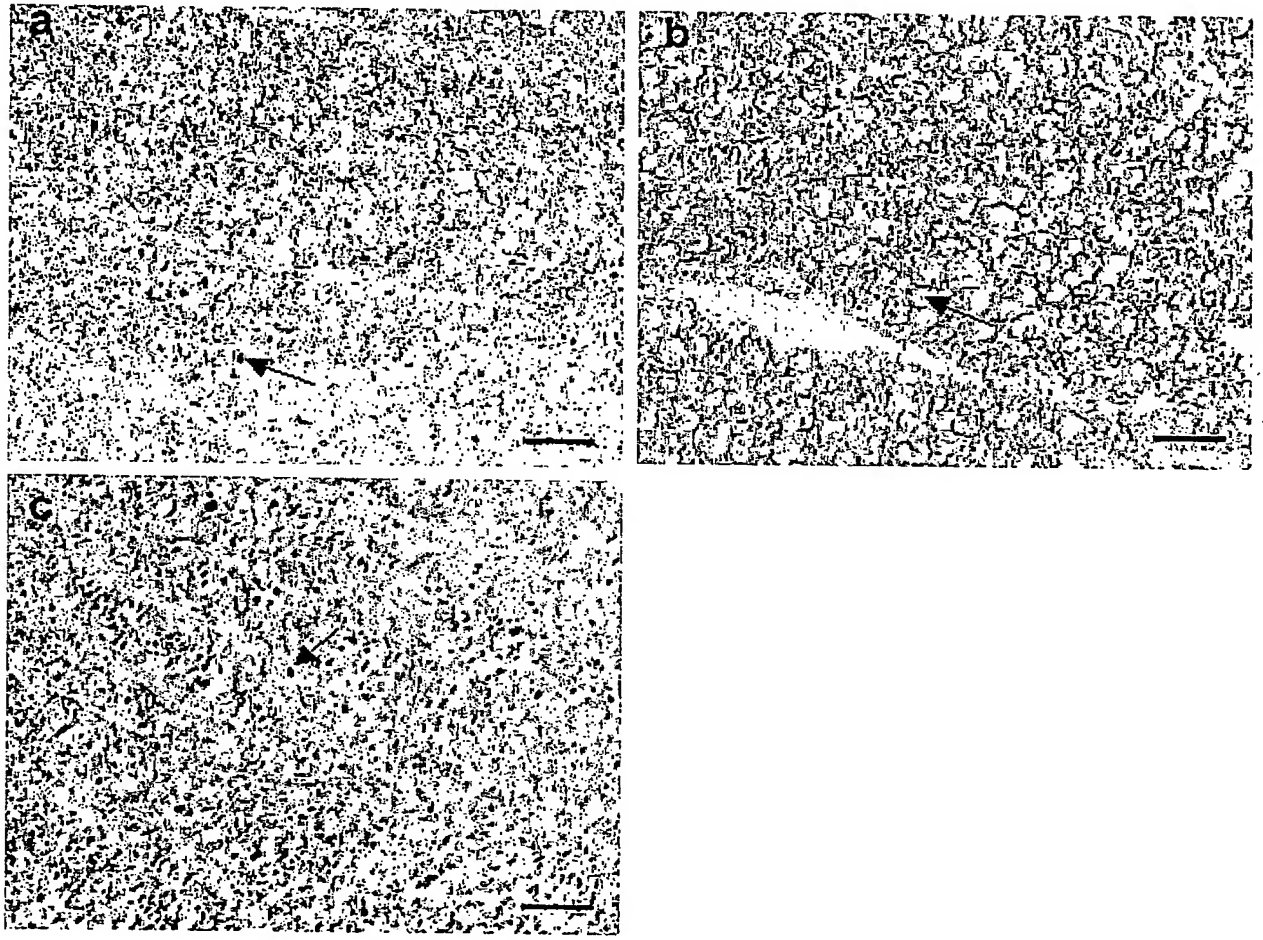


Figure 24A and 24B

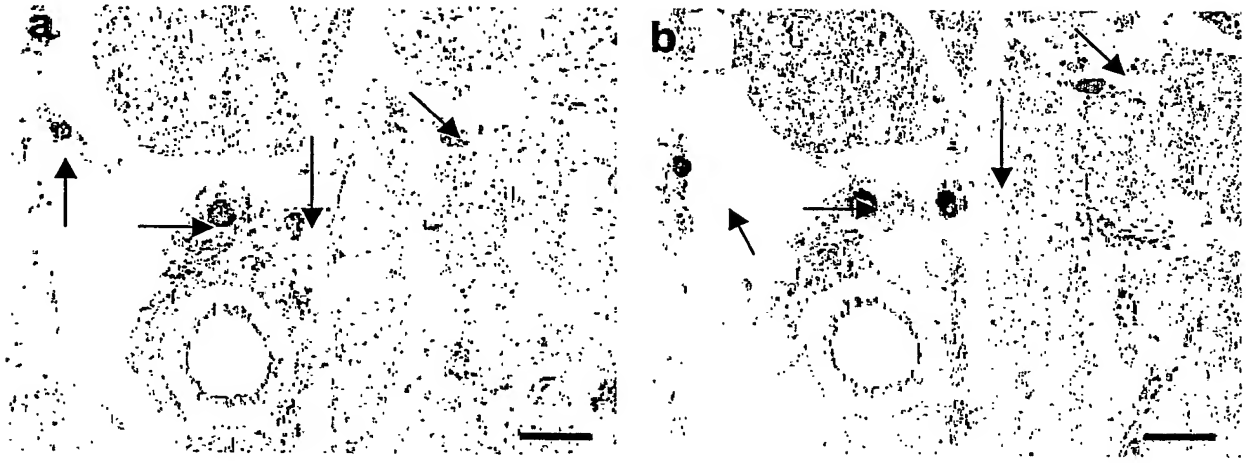


Figure 25A, 25B, 25C and 25D

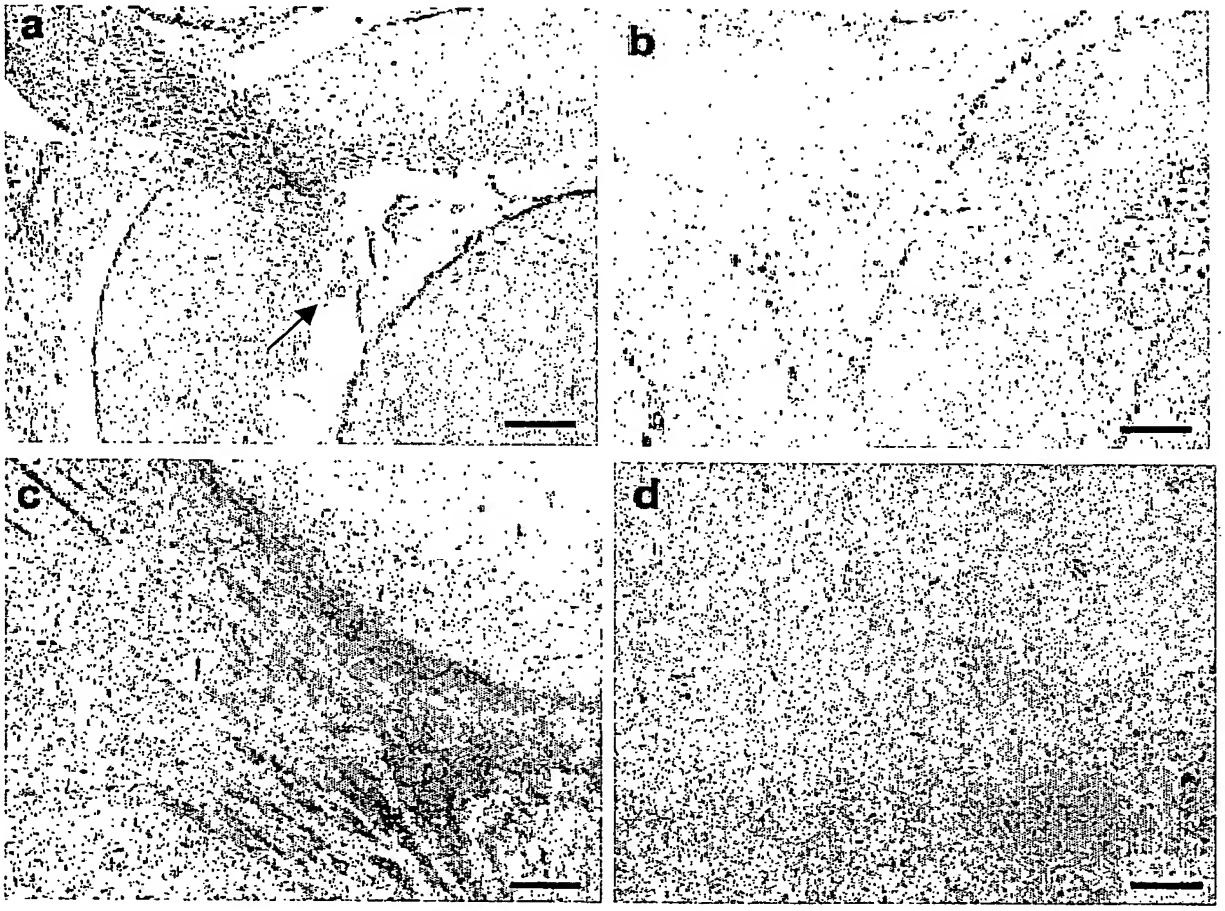


Figure 26A and 26B

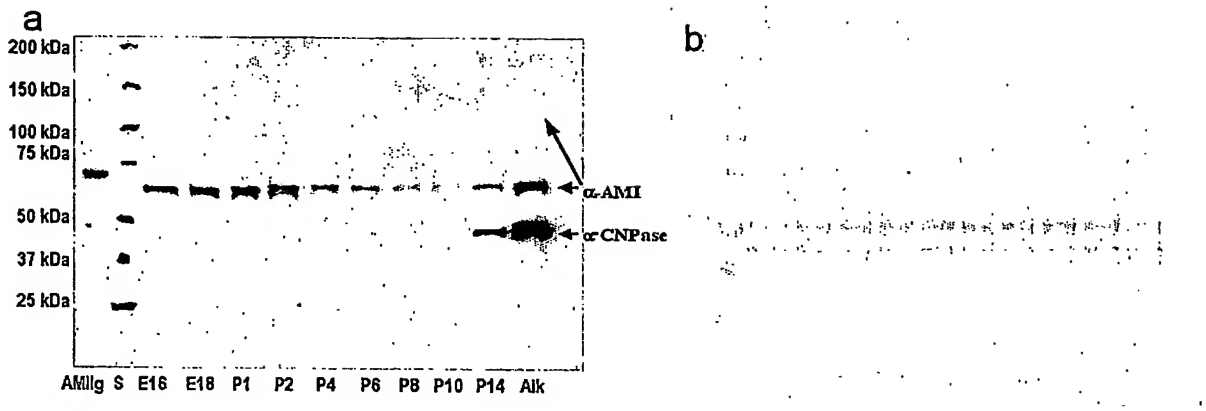


Figure 27

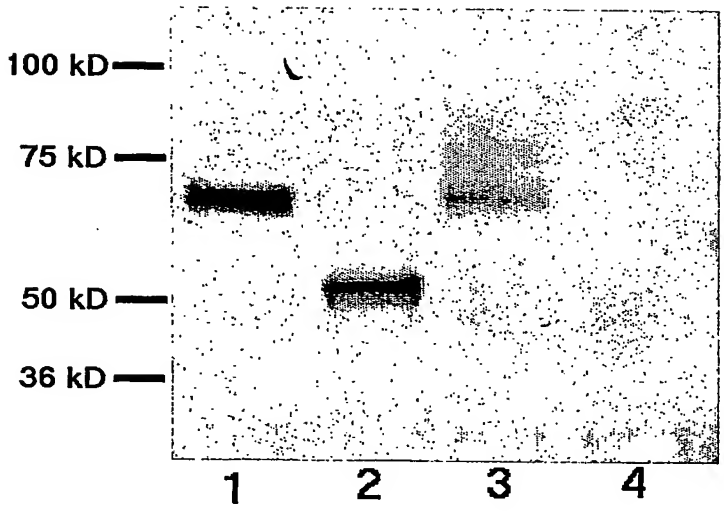


Figure 28

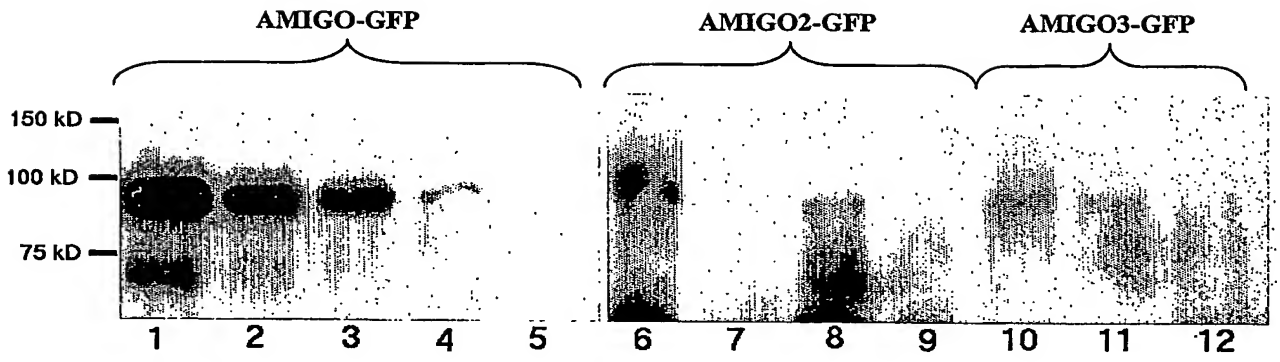
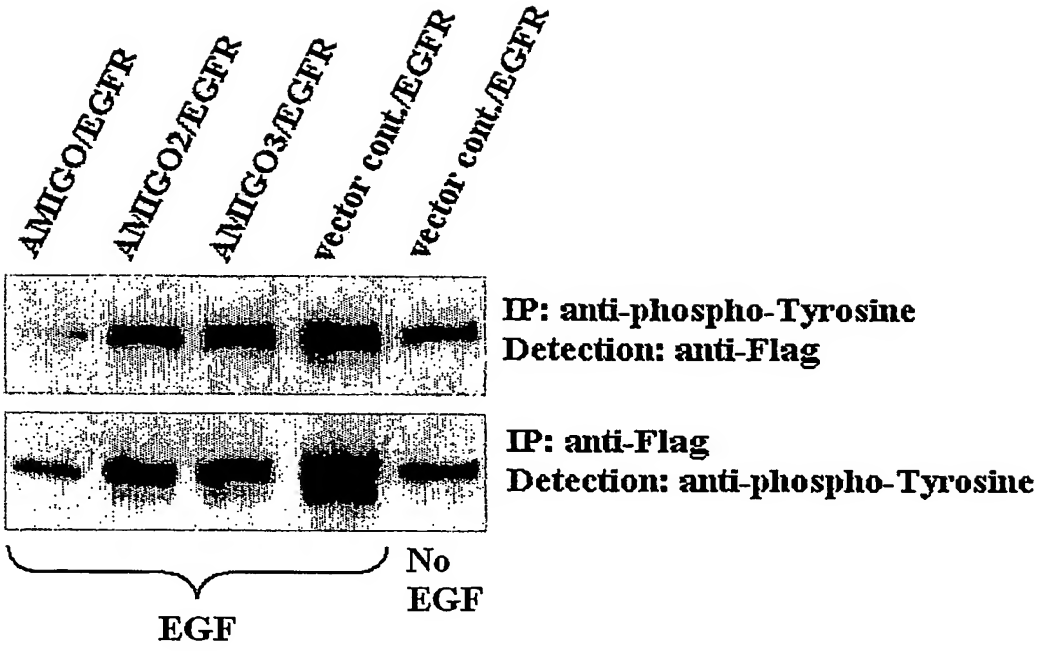

AMIGO-GFP
AMIGO2-GFP
AMIGO3-GFP
150 kD
100 kD
75 kD
1
2
3
4
5
6
7
8
9
10
11
12

Figure 29


AMIGO/EGFR
AMIGO2/EGFR
AMIGO3/EGFR
vector cont./EGFR
vector cont./EGFR
IP: anti-phospho-Tyrosine
Detection: anti-Flag
IP: anti-Flag
Detection: anti-phospho-Tyrosine
No EGF
EGF

SEQUENCE LISTING

<110> Licentia Ltd.

<120> Novel protein and uses thereof

<130> 40552

<150> US 60/433,011
<151> 2002-12-13

<160> 32

<170> PatentIn Ver. 2.1

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<400> 1

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Met His Pro His Arg Asp Pro Arg Gly Leu Trp Leu Leu Leu Pro Ser
  1               5                  10                  15

ttg tcc ctg ctg ctt ttt gag gtg gcc aga gct ggc cga gcc gtg gtt   96
Leu Ser Leu Leu Leu Phe Glu Val Ala Arg Ala Gly Arg Ala Val Val
             20                  25                  30

agc tgt cct gcc gcc tgc ttg tgc gcc agc aac atc ctc agc tgc tcc   144
Ser Cys Pro Ala Ala Cys Leu Cys Ala Ser Asn Ile Leu Ser Cys Ser
         35                  40                  45

aag cag cag ctg ccc aat gtg ccc cat tcc ttg ccc agt tac aca gca   192
Lys Gln Gln Leu Pro Asn Val Pro His Ser Leu Pro Ser Tyr Thr Ala
     50                  55                  60

cta ctg gac ctc agt cac aac aac ctg agc cgc ctg cgg gcc gag tgg   240
Leu Leu Asp Leu Ser His Asn Asn Leu Ser Arg Leu Arg Ala Glu Trp
 65                  70                  75                  80

acc ccc acg cgc ctg acc caa ctg cac tcc ctg ctg ctg agc cac aac   288
Thr Pro Thr Arg Leu Thr Gln Leu His Ser Leu Leu Leu Ser His Asn
                 85                  90                  95

cac ctg aac ttc atc tcc tct gag gcc ttt tcc ccg gta ccc aac ctg   336
His Leu Asn Phe Ile Ser Ser Glu Ala Phe Ser Pro Val Pro Asn Leu
            100                 105                 110

cgc tac ctg gac ctc tcc tcc aac cag ctg cgt aca ctg gat gag ttc   384
Arg Tyr Leu Asp Leu Ser Ser Asn Gln Leu Arg Thr Leu Asp Glu Phe
        115                 120                 125

ctg ttc agt gac ctg caa gta ctg gag gtg ctg ctg ctc tac aat aac   432
Leu Phe Ser Asp Leu Gln Val Leu Glu Val Leu Leu Leu Tyr Asn Asn
    130                 135                 140
```

```
cac atc atg gcg gtg gac cgg tgc gcc ttc gat gac atg gcc cag ctg   480
His Ile Met Ala Val Asp Arg Cys Ala Phe Asp Asp Met Ala Gln Leu
145                 150                 155                 160

cag aaa ctc tac ttg agc cag aac cag atc tct cgc ttc cct ctg gaa   528
Gln Lys Leu Tyr Leu Ser Gln Asn Gln Ile Ser Arg Phe Pro Leu Glu
                165                 170                 175

ctg gtc aag gaa gga gcc aag cta ccc aaa cta acg ctc ctg gat ctc   576
Leu Val Lys Glu Gly Ala Lys Leu Pro Lys Leu Thr Leu Leu Asp Leu
            180                 185                 190

tct tct aac aag ctg aag aac ttg cca ttg cct gac ctg cag aag ctg   624
Ser Ser Asn Lys Leu Lys Asn Leu Pro Leu Pro Asp Leu Gln Lys Leu
        195                 200                 205

ccg gcc tgg atc aag aat ggg ctg tac cta cat aac aac ccc ctg aac   672
Pro Ala Trp Ile Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Asn
    210                 215                 220

tgc gac tgt gag ctc tac cag ctg ttt tca cac tgg cag tat cgg cag   720
Cys Asp Cys Glu Leu Tyr Gln Leu Phe Ser His Trp Gln Tyr Arg Gln
225                 230                 235                 240

ctg agc tcc gtg atg gac ttt caa gag gat ctg tac tgc atg aac tcc   768
Leu Ser Ser Val Met Asp Phe Gln Glu Asp Leu Tyr Cys Met Asn Ser
                245                 250                 255

aag aag ctg cac aat gtc ttc aac ctg agt ttc ctc aac tgt ggc gag   816
Lys Lys Leu His Asn Val Phe Asn Leu Ser Phe Leu Asn Cys Gly Glu
            260                 265                 270

tac aag gag cgt gcc tgg gag gcc cac ctg ggt gac acc ttg atc atc   864
Tyr Lys Glu Arg Ala Trp Glu Ala His Leu Gly Asp Thr Leu Ile Ile
        275                 280                 285

aag tgt gac acc aag cag caa ggg atg acc aag gtg tgg gtg aca cca   912
Lys Cys Asp Thr Lys Gln Gln Gly Met Thr Lys Val Trp Val Thr Pro
    290                 295                 300

agt aat gaa cgg gtg cta gat gag gtg acc aat ggc aca gtg agt gtg   960
Ser Asn Glu Arg Val Leu Asp Glu Val Thr Asn Gly Thr Val Ser Val
305                 310                 315                 320

tct aag gat ggc agt ctt ctt ttc cag cag gtg cag gtc gag gac ggt  1008
Ser Lys Asp Gly Ser Leu Leu Phe Gln Gln Val Gln Val Glu Asp Gly
                325                 330                 335

ggt gtg tat acc tgc tat gcc atg gga gag act ttc aat gag aca ctg  1056
Gly Val Tyr Thr Cys Tyr Ala Met Gly Glu Thr Phe Asn Glu Thr Leu
            340                 345                 350

tct gtg gaa ttg aaa gtg cac aat ttc acc ttg cac gga cac cat gac  1104
Ser Val Glu Leu Lys Val His Asn Phe Thr Leu His Gly His His Asp
        355                 360                 365

acc ctc aac aca gcc tat acc acc cta gtg ggc tgt atc ctt agt gtg  1152
Thr Leu Asn Thr Ala Tyr Thr Thr Leu Val Gly Cys Ile Leu Ser Val
    370                 375                 380

gtc ctg gtc ctc ata tac cta tac ctc acc cct tgc cgc tgc tgg tgc  1200
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```
Val Leu Val Leu Ile Tyr Leu Tyr Leu Thr Pro Cys Arg Cys Trp Cys
385                 390                 395                 400

cgg ggt gta gag aag cct tcc agc cat caa gga gac agc ctc agc tct   1248
Arg Gly Val Glu Lys Pro Ser Ser His Gln Gly Asp Ser Leu Ser Ser
                405                 410                 415

tcc atg ctt agt acc aca ccc aac cat gat cct atg gct ggt ggg gac   1296
Ser Met Leu Ser Thr Thr Pro Asn His Asp Pro Met Ala Gly Gly Asp
            420                 425                 430

aaa gat gat ggt ttt gac cgg cgg gtg gct ttc ctg gaa cct gct gga   1344
Lys Asp Asp Gly Phe Asp Arg Arg Val Ala Phe Leu Glu Pro Ala Gly
        435                 440                 445

cct ggg cag ggt caa aac ggc aag ctc aag cca ggc aac acc ctg cca   1392
Pro Gly Gln Gly Gln Asn Gly Lys Leu Lys Pro Gly Asn Thr Leu Pro
    450                 455                 460

gtg cct gag gcc aca ggc aag ggc caa cgg agg atg tcg gat cca gaa   1440
Val Pro Glu Ala Thr Gly Lys Gly Gln Arg Arg Met Ser Asp Pro Glu
465                 470                 475                 480

tca gtc agc tcg gtc ttc tct gat acg ccc att gtg gtg               1479
Ser Val Ser Ser Val Phe Ser Asp Thr Pro Ile Val Val
                485                 490


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<212> PRT
<213> Homo sapiens

<400> 2
Met His Pro His Arg Asp Pro Arg Gly Leu Trp Leu Leu Leu Pro Ser
  1               5                  10                  15

Leu Ser Leu Leu Leu Phe Glu Val Ala Arg Ala Gly Arg Ala Val Val
             20                  25                  30

Ser Cys Pro Ala Ala Cys Leu Cys Ala Ser Asn Ile Leu Ser Cys Ser
         35                  40                  45

Lys Gln Gln Leu Pro Asn Val Pro His Ser Leu Pro Ser Tyr Thr Ala
     50                  55                  60

Leu Leu Asp Leu Ser His Asn Asn Leu Ser Arg Leu Arg Ala Glu Trp
 65                  70                  75                  80

Thr Pro Thr Arg Leu Thr Gln Leu His Ser Leu Leu Leu Ser His Asn
                 85                  90                  95

His Leu Asn Phe Ile Ser Ser Glu Ala Phe Ser Pro Val Pro Asn Leu
            100                 105                 110

Arg Tyr Leu Asp Leu Ser Ser Asn Gln Leu Arg Thr Leu Asp Glu Phe
        115                 120                 125

Leu Phe Ser Asp Leu Gln Val Leu Glu Val Leu Leu Leu Tyr Asn Asn
    130                 135                 140
```

```
His Ile Met Ala Val Asp Arg Cys Ala Phe Asp Asp Met Ala Gln Leu
145                 150                 155                 160

Gln Lys Leu Tyr Leu Ser Gln Asn Gln Ile Ser Arg Phe Pro Leu Glu
                165                 170                 175

Leu Val Lys Glu Gly Ala Lys Leu Pro Lys Leu Thr Leu Leu Asp Leu
            180                 185                 190

Ser Ser Asn Lys Leu Lys Asn Leu Pro Leu Pro Asp Leu Gln Lys Leu
        195                 200                 205

Pro Ala Trp Ile Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Asn
    210                 215                 220

Cys Asp Cys Glu Leu Tyr Gln Leu Phe Ser His Trp Gln Tyr Arg Gln
225                 230                 235                 240

Leu Ser Ser Val Met Asp Phe Gln Glu Asp Leu Tyr Cys Met Asn Ser
                245                 250                 255

Lys Lys Leu His Asn Val Phe Asn Leu Ser Phe Leu Asn Cys Gly Glu
            260                 265                 270

Tyr Lys Glu Arg Ala Trp Glu Ala His Leu Gly Asp Thr Leu Ile Ile
        275                 280                 285

Lys Cys Asp Thr Lys Gln Gln Gly Met Thr Lys Val Trp Val Thr Pro
    290                 295                 300

Ser Asn Glu Arg Val Leu Asp Glu Val Thr Asn Gly Thr Val Ser Val
305                 310                 315                 320

Ser Lys Asp Gly Ser Leu Leu Phe Gln Gln Val Gln Val Glu Asp Gly
                325                 330                 335

Gly Val Tyr Thr Cys Tyr Ala Met Gly Glu Thr Phe Asn Glu Thr Leu
            340                 345                 350

Ser Val Glu Leu Lys Val His Asn Phe Thr Leu His Gly His His Asp
        355                 360                 365

Thr Leu Asn Thr Ala Tyr Thr Thr Leu Val Gly Cys Ile Leu Ser Val
    370                 375                 380

Val Leu Val Leu Ile Tyr Leu Tyr Leu Thr Pro Cys Arg Cys Trp Cys
385                 390                 395                 400

Arg Gly Val Glu Lys Pro Ser Ser His Gln Gly Asp Ser Leu Ser Ser
                405                 410                 415

Ser Met Leu Ser Thr Thr Pro Asn His Asp Pro Met Ala Gly Gly Asp
            420                 425                 430

Lys Asp Asp Gly Phe Asp Arg Arg Val Ala Phe Leu Glu Pro Ala Gly
        435                 440                 445

Pro Gly Gln Gly Gln Asn Gly Lys Leu Lys Pro Gly Asn Thr Leu Pro
    450                 455                 460

Val Pro Glu Ala Thr Gly Lys Gly Gln Arg Arg Met Ser Asp Pro Glu
```

```
465                 470                 475                 480

Ser Val Ser Ser Val Phe Ser Asp Thr Pro Ile Val Val
                485                 490
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<210> 3
<211> 1566
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1566)

<400> 3

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atg tcg tta cgt gta cac act ctg ccc acc ctg ctt gga gcc gtc gtc   48
Met Ser Leu Arg Val His Thr Leu Pro Thr Leu Leu Gly Ala Val Val
  1               5                  10                  15

aga ccg ggc tgc agg gag ctg ctg tgt ttg ctg atg atc aca gtg act   96
Arg Pro Gly Cys Arg Glu Leu Leu Cys Leu Leu Met Ile Thr Val Thr
            20                  25                  30

gtg ggc cct ggt gcc tct ggg gtg tgc ccc acc gct tgc atc tgt gcc   144
Val Gly Pro Gly Ala Ser Gly Val Cys Pro Thr Ala Cys Ile Cys Ala
        35                  40                  45

act gac atc gtc agc tgc acc aac aaa aac ctg tcc aag gtg cct ggg   192
Thr Asp Ile Val Ser Cys Thr Asn Lys Asn Leu Ser Lys Val Pro Gly
    50                  55                  60

aac ctt ttc aga ctg att aag aga ctg gac ctg agt tat aac aga att   240
Asn Leu Phe Arg Leu Ile Lys Arg Leu Asp Leu Ser Tyr Asn Arg Ile
65                  70                  75                  80

ggg ctt ctg gat tct gag tgg att cca gta tcg ttt gca aag ctg aac   288
Gly Leu Leu Asp Ser Glu Trp Ile Pro Val Ser Phe Ala Lys Leu Asn
                85                  90                  95

acc cta att ctt cgt cat aac aac atc acc agc att tcc acg ggc agt   336
Thr Leu Ile Leu Arg His Asn Asn Ile Thr Ser Ile Ser Thr Gly Ser
            100                 105                 110

ttt tcc aca act cca aat ttg aag tgt ctt gac tta tcg tcc aat aag   384
Phe Ser Thr Thr Pro Asn Leu Lys Cys Leu Asp Leu Ser Ser Asn Lys
        115                 120                 125

ctg aag acg gtg aaa aat gct gta ttc caa gag ttg aag gtt ctg gaa   432
Leu Lys Thr Val Lys Asn Ala Val Phe Gln Glu Leu Lys Val Leu Glu
    130                 135                 140

gtg ctt ctg ctt tac aac aat cac ata tcc tat ctc gat cct tca gcg   480
Val Leu Leu Leu Tyr Asn Asn His Ile Ser Tyr Leu Asp Pro Ser Ala
145                 150                 155                 160

ttt gga ggg ctc tcc cag ttg cag aaa ctc tac tta agt gga aat ttt   528
Phe Gly Gly Leu Ser Gln Leu Gln Lys Leu Tyr Leu Ser Gly Asn Phe
                165                 170                 175
```

```
ctc aca cag ttt ccg atg gat ttg tat gtt gga agg ttc aag ctg gca   576
Leu Thr Gln Phe Pro Met Asp Leu Tyr Val Gly Arg Phe Lys Leu Ala
            180                 185                 190

gaa ctg atg ttt tta gat gtt tct tat aac cga att cct tcc atg cca   624
Glu Leu Met Phe Leu Asp Val Ser Tyr Asn Arg Ile Pro Ser Met Pro
        195                 200                 205

atg cac cac ata aat tta gtg cca gga aaa cag ctg aga ggc atc tac   672
Met His His Ile Asn Leu Val Pro Gly Lys Gln Leu Arg Gly Ile Tyr
    210                 215                 220

ctt cat gga aac cca ttt gtc tgt gac tgt tcc ctg tac tcc ttg ctg   720
Leu His Gly Asn Pro Phe Val Cys Asp Cys Ser Leu Tyr Ser Leu Leu
225                 230                 235                 240

gtc ttt tgg tat cgt agg cac ttt agc tca gtg atg gat ttt aag aac   768
Val Phe Trp Tyr Arg Arg His Phe Ser Ser Val Met Asp Phe Lys Asn
                245                 250                 255

gat tac acc tgt cgc ctg tgg tct gac tcc agg cac tcg cgt cag gta   816
Asp Tyr Thr Cys Arg Leu Trp Ser Asp Ser Arg His Ser Arg Gln Val
            260                 265                 270

ctt ctg ctc cag gat agc ttt atg aat tgc tct gac agc atc atc aat   864
Leu Leu Leu Gln Asp Ser Phe Met Asn Cys Ser Asp Ser Ile Ile Asn
        275                 280                 285

ggt tcc ttt cgt gcg ctt ggc ttt att cat gag gct cag gtc ggg gaa   912
Gly Ser Phe Arg Ala Leu Gly Phe Ile His Glu Ala Gln Val Gly Glu
    290                 295                 300

aga ctg atg gtc cac tgt gac agc aag aca ggt aat gca aat acg gat   960
Arg Leu Met Val His Cys Asp Ser Lys Thr Gly Asn Ala Asn Thr Asp
305                 310                 315                 320

ttc atc tgg gtg ggt cca gat aac aga ctg cta gag ccg gat aaa gag   1008
Phe Ile Trp Val Gly Pro Asp Asn Arg Leu Leu Glu Pro Asp Lys Glu
                325                 330                 335

atg gaa aac ttt tac gtg ttt cac aat gga agt ctg gtt ata gaa agc   1056
Met Glu Asn Phe Tyr Val Phe His Asn Gly Ser Leu Val Ile Glu Ser
            340                 345                 350

cct cgt ttt gag gat gct gga gtg tat tct tgt atc gca atg aat aag   1104
Pro Arg Phe Glu Asp Ala Gly Val Tyr Ser Cys Ile Ala Met Asn Lys
        355                 360                 365

caa cgc ctg tta aat gaa act gtg gac gtc aca ata aat gtg agc aat   1152
Gln Arg Leu Leu Asn Glu Thr Val Asp Val Thr Ile Asn Val Ser Asn
    370                 375                 380

ttc act gta agc aga tcc cat gct cat gag gca ttt aac aca gct ttt   1200
Phe Thr Val Ser Arg Ser His Ala His Glu Ala Phe Asn Thr Ala Phe
385                 390                 395                 400

acc act ctt gct gct tgc gtg gcc agt atc gtt ttg gta ctt ttg tac   1248
Thr Thr Leu Ala Ala Cys Val Ala Ser Ile Val Leu Val Leu Leu Tyr
                405                 410                 415

ctc tat ctg act cca tgc ccc tgc aag tgt aaa acc aag aga cag aaa   1296
```

```
Leu Tyr Leu Thr Pro Cys Pro Cys Lys Cys Lys Thr Lys Arg Gln Lys
            420                 425                 430

aat atg cta cac caa agc aat gcc cat tca tcg att ctc agt cct ggc   1344
Asn Met Leu His Gln Ser Asn Ala His Ser Ser Ile Leu Ser Pro Gly
        435                 440                 445

ccc gct agt gat gcc tcc gct gat gaa cgg aag gca ggt gca ggt aaa   1392
Pro Ala Ser Asp Ala Ser Ala Asp Glu Arg Lys Ala Gly Ala Gly Lys
    450                 455                 460

aga gtg gtg ttt ttg gaa ccc ctg aag gat act gca gca ggg cag aac   1440
Arg Val Val Phe Leu Glu Pro Leu Lys Asp Thr Ala Ala Gly Gln Asn
465                 470                 475                 480

ggg aaa gtc agg ctc ttt ccc agc gag gca gtg ata gct gag ggc atc   1488
Gly Lys Val Arg Leu Phe Pro Ser Glu Ala Val Ile Ala Glu Gly Ile
                485                 490                 495

cta aag tcc acg agg ggg aaa tct gac tca gat tca gtc aat tca gtg   1536
Leu Lys Ser Thr Arg Gly Lys Ser Asp Ser Asp Ser Val Asn Ser Val
            500                 505                 510

ttt tct gac aca cct ttt gtg gcg tcc act                          1566
Phe Ser Asp Thr Pro Phe Val Ala Ser Thr
        515                 520
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<210> 4
<211> 522
<212> PRT
<213> Homo sapiens

<400> 4

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Met Ser Leu Arg Val His Thr Leu Pro Thr Leu Leu Gly Ala Val Val
  1               5                  10                  15

Arg Pro Gly Cys Arg Glu Leu Leu Cys Leu Leu Met Ile Thr Val Thr
             20                  25                  30

Val Gly Pro Gly Ala Ser Gly Val Cys Pro Thr Ala Cys Ile Cys Ala
         35                  40                  45

Thr Asp Ile Val Ser Cys Thr Asn Lys Asn Leu Ser Lys Val Pro Gly
     50                  55                  60

Asn Leu Phe Arg Leu Ile Lys Arg Leu Asp Leu Ser Tyr Asn Arg Ile
 65                  70                  75                  80

Gly Leu Leu Asp Ser Glu Trp Ile Pro Val Ser Phe Ala Lys Leu Asn
                 85                  90                  95

Thr Leu Ile Leu Arg His Asn Asn Ile Thr Ser Ile Ser Thr Gly Ser
            100                 105                 110

Phe Ser Thr Thr Pro Asn Leu Lys Cys Leu Asp Leu Ser Ser Asn Lys
        115                 120                 125

Leu Lys Thr Val Lys Asn Ala Val Phe Gln Glu Leu Lys Val Leu Glu
    130                 135                 140
```

Val Leu Leu Leu Tyr Asn Asn His Ile Ser Tyr Leu Asp Pro Ser Ala
145 150 155 160

Phe Gly Gly Leu Ser Gln Leu Gln Lys Leu Tyr Leu Ser Gly Asn Phe
165 170 175

Leu Thr Gln Phe Pro Met Asp Leu Tyr Val Gly Arg Phe Lys Leu Ala
180 185 190

Glu Leu Met Phe Leu Asp Val Ser Tyr Asn Arg Ile Pro Ser Met Pro
195 200 205

Met His His Ile Asn Leu Val Pro Gly Lys Gln Leu Arg Gly Ile Tyr
210 215 220

Leu His Gly Asn Pro Phe Val Cys Asp Cys Ser Leu Tyr Ser Leu Leu
225 230 235 240

Val Phe Trp Tyr Arg Arg His Phe Ser Ser Val Met Asp Phe Lys Asn
245 250 255

Asp Tyr Thr Cys Arg Leu Trp Ser Asp Ser Arg His Ser Arg Gln Val
260 265 270

Leu Leu Leu Gln Asp Ser Phe Met Asn Cys Ser Asp Ser Ile Ile Asn
275 280 285

Gly Ser Phe Arg Ala Leu Gly Phe Ile His Glu Ala Gln Val Gly Glu
290 295 300

Arg Leu Met Val His Cys Asp Ser Lys Thr Gly Asn Ala Asn Thr Asp
305 310 315 320

Phe Ile Trp Val Gly Pro Asp Asn Arg Leu Leu Glu Pro Asp Lys Glu
325 330 335

Met Glu Asn Phe Tyr Val Phe His Asn Gly Ser Leu Val Ile Glu Ser
340 345 350

Pro Arg Phe Glu Asp Ala Gly Val Tyr Ser Cys Ile Ala Met Asn Lys
355 360 365

Gln Arg Leu Leu Asn Glu Thr Val Asp Val Thr Ile Asn Val Ser Asn
370 375 380

Phe Thr Val Ser Arg Ser His Ala His Glu Ala Phe Asn Thr Ala Phe
385 390 395 400

Thr Thr Leu Ala Ala Cys Val Ala Ser Ile Val Leu Val Leu Leu Tyr
405 410 415

Leu Tyr Leu Thr Pro Cys Pro Cys Lys Cys Lys Thr Lys Arg Gln Lys
420 425 430

Asn Met Leu His Gln Ser Asn Ala His Ser Ser Ile Leu Ser Pro Gly
435 440 445

Pro Ala Ser Asp Ala Ser Ala Asp Glu Arg Lys Ala Gly Ala Gly Lys
450 455 460

Arg Val Val Phe Leu Glu Pro Leu Lys Asp Thr Ala Ala Gly Gln Asn

```
465                 470                 475                 480

Gly Lys Val Arg Leu Phe Pro Ser Glu Ala Val Ile Ala Glu Gly Ile
                485                 490                 495

Leu Lys Ser Thr Arg Gly Lys Ser Asp Ser Asp Ser Val Asn Ser Val
            500                 505                 510

Phe Ser Asp Thr Pro Phe Val Ala Ser Thr
        515                 520



<210> 5
<211> 1512
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1512)

<400> 5
atg acc tgg ttg gtg ctg ctg ggg aca ctg ctc tgc atg ctg cgc gtt   48
Met Thr Trp Leu Val Leu Leu Gly Thr Leu Leu Cys Met Leu Arg Val
  1               5                  10                  15

ggg tta ggc acc ccg gac tcc gag ggt ttc ccg ccc cgt gcg ctc cac   96
Gly Leu Gly Thr Pro Asp Ser Glu Gly Phe Pro Pro Arg Ala Leu His
            20                  25                  30

aac tgc ccc tac aaa tgt atc tgc gct gcc gac ctg cta agc tgc act   144
Asn Cys Pro Tyr Lys Cys Ile Cys Ala Ala Asp Leu Leu Ser Cys Thr
        35                  40                  45

ggc cta ggg ctg cag gac gtg cca gcc gag tta cct gcc gct act gcg   192
Gly Leu Gly Leu Gln Asp Val Pro Ala Glu Leu Pro Ala Ala Thr Ala
    50                  55                  60

gac ctc gac ctg agc cac aac gcg ctc cag cgc ctg cgc ccc ggc tgg   240
Asp Leu Asp Leu Ser His Asn Ala Leu Gln Arg Leu Arg Pro Gly Trp
65                  70                  75                  80

ttg gcg ccc ctc ttc cag ctg cgc gcc ctg cac cta gac cac aac gaa   288
Leu Ala Pro Leu Phe Gln Leu Arg Ala Leu His Leu Asp His Asn Glu
                85                  90                  95

cta gat gcg ctg ggt cgc ggc gtc ttc gtc aac gcc agc ggc ctg agg   336
Leu Asp Ala Leu Gly Arg Gly Val Phe Val Asn Ala Ser Gly Leu Arg
            100                 105                 110

ctg ctc gat cta tca tct aac acg ttg cgg gcg ctt ggc cgc cac gac   384
Leu Leu Asp Leu Ser Ser Asn Thr Leu Arg Ala Leu Gly Arg His Asp
        115                 120                 125

ctc gac ggg ctg ggg gcg ctg gag aag ctg ctt ctg ttc aat aac cgc   432
Leu Asp Gly Leu Gly Ala Leu Glu Lys Leu Leu Leu Phe Asn Asn Arg
    130                 135                 140

ttg gtg cac ttg gac gag cat gcc ttc cac ggc ctg cgc gcg ctc agc   480
Leu Val His Leu Asp Glu His Ala Phe His Gly Leu Arg Ala Leu Ser
```

```
145                 150                 155                 160

cat ctc tac ctg ggc tgc aac gaa ctc gcc tcg ttc tcc ttc gac cac   528
His Leu Tyr Leu Gly Cys Asn Glu Leu Ala Ser Phe Ser Phe Asp His
                165                 170                 175

ctg cac ggt ctg agc gcc acc cac ctg ctt act ctg gac ctc tcc tcc   576
Leu His Gly Leu Ser Ala Thr His Leu Leu Thr Leu Asp Leu Ser Ser
            180                 185                 190

aac cgg ctg gga cac atc tcc gta cct gag ctg gcc gcg ctg ccg gcc   624
Asn Arg Leu Gly His Ile Ser Val Pro Glu Leu Ala Ala Leu Pro Ala
        195                 200                 205

ttc ctc aag aac ggc ctc tac ttg cac aac aac cct ttg cct tgc gac   672
Phe Leu Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Pro Cys Asp
    210                 215                 220

tgc cgc ctc tac cac ctg cta cag cgc tgg cac cag cgg ggc ctg agc   720
Cys Arg Leu Tyr His Leu Leu Gln Arg Trp His Gln Arg Gly Leu Ser
225                 230                 235                 240

gcc gtg cgc gac ttt gcg cgc gag tac gta tgc ttg gcc ttc aag gta   768
Ala Val Arg Asp Phe Ala Arg Glu Tyr Val Cys Leu Ala Phe Lys Val
                245                 250                 255

ccc gcg tcc cgc gtg cgc ttc ttc cag cac agc cgc gtc ttt gag aac   816
Pro Ala Ser Arg Val Arg Phe Phe Gln His Ser Arg Val Phe Glu Asn
            260                 265                 270

tgc tcg tcg gcc cca gct ctt ggc cta gag cgg ccg gaa gag cac ctg   864
Cys Ser Ser Ala Pro Ala Leu Gly Leu Glu Arg Pro Glu Glu His Leu
        275                 280                 285

tac gcg ctg gtg ggt cgg tcc ctg agg ctt tac tgc aac acc agc gtc   912
Tyr Ala Leu Val Gly Arg Ser Leu Arg Leu Tyr Cys Asn Thr Ser Val
    290                 295                 300

ccg gcc atg cgc att gcc tgg gtt tcg ccg cag cag gag ctt ctc agg   960
Pro Ala Met Arg Ile Ala Trp Val Ser Pro Gln Gln Glu Leu Leu Arg
305                 310                 315                 320

gcg cca gga tcc cgc gat ggc agc atc gcg gtg ctg gcc gac ggc agc   1008
Ala Pro Gly Ser Arg Asp Gly Ser Ile Ala Val Leu Ala Asp Gly Ser
                325                 330                 335

ttg gcc ata ggc aac gta cag gag cag cat gcg gga ctc ttc gtg tgc   1056
Leu Ala Ile Gly Asn Val Gln Glu Gln His Ala Gly Leu Phe Val Cys
            340                 345                 350

ctg gcc act ggg ccc cgc ctg cac cac aac cag acg cac gag tac aac   1104
Leu Ala Thr Gly Pro Arg Leu His His Asn Gln Thr His Glu Tyr Asn
        355                 360                 365

gtg agc gtg cac ttt ccg cgc cca gag ccc gag gct ttc aac aca ggc   1152
Val Ser Val His Phe Pro Arg Pro Glu Pro Glu Ala Phe Asn Thr Gly
    370                 375                 380

ttc acc aca ctg ctg ggc tgt gcc gtg ggc ctt gtg ctc gtg ctg ctc   1200
Phe Thr Thr Leu Leu Gly Cys Ala Val Gly Leu Val Leu Val Leu Leu
385                 390                 395                 400
```

```
tac ctg ttc gcc cca ccc tgc cgc tgc tgc cgc cgt gcc tgc cgc tgc   1248
Tyr Leu Phe Ala Pro Pro Cys Arg Cys Cys Arg Arg Ala Cys Arg Cys
                405                 410                 415

cgc cgc tgg ccc caa aca ccc agc ccg ctc caa gag ctg agc gca cag   1296
Arg Arg Trp Pro Gln Thr Pro Ser Pro Leu Gln Glu Leu Ser Ala Gln
            420                 425                 430

tcc tca gta ctc agc acc aca ccg cca gac gca ccc agc cgc aag gcc   1344
Ser Ser Val Leu Ser Thr Thr Pro Pro Asp Ala Pro Ser Arg Lys Ala
        435                 440                 445

agc gtc cac aag cac gta gtc ttt ctg gag cca ggc cgg agg ggc ctc   1392
Ser Val His Lys His Val Val Phe Leu Glu Pro Gly Arg Arg Gly Leu
    450                 455                 460

aat ggc cgc gtg cag ctg gca gta gct gag gaa ttc gat ctc tac aac   1440
Asn Gly Arg Val Gln Leu Ala Val Ala Glu Glu Phe Asp Leu Tyr Asn
465                 470                 475                 480

cct gga ggc ctg cag ctg aag gct ggc tct gag tcc gcc agc tcc ata   1488
Pro Gly Gly Leu Gln Leu Lys Ala Gly Ser Glu Ser Ala Ser Ser Ile
                485                 490                 495

ggc tcc gag ggt ccc atg aca acc                                   1512
Gly Ser Glu Gly Pro Met Thr Thr
            500
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<210> 6
<211> 504
<212> PRT
<213> Homo sapiens

<400> 6

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Met Thr Trp Leu Val Leu Leu Gly Thr Leu Leu Cys Met Leu Arg Val
  1               5                  10                  15

Gly Leu Gly Thr Pro Asp Ser Glu Gly Phe Pro Pro Arg Ala Leu His
             20                  25                  30

Asn Cys Pro Tyr Lys Cys Ile Cys Ala Ala Asp Leu Leu Ser Cys Thr
         35                  40                  45

Gly Leu Gly Leu Gln Asp Val Pro Ala Glu Leu Pro Ala Ala Thr Ala
     50                  55                  60

Asp Leu Asp Leu Ser His Asn Ala Leu Gln Arg Leu Arg Pro Gly Trp
 65                  70                  75                  80

Leu Ala Pro Leu Phe Gln Leu Arg Ala Leu His Leu Asp His Asn Glu
                 85                  90                  95

Leu Asp Ala Leu Gly Arg Gly Val Phe Val Asn Ala Ser Gly Leu Arg
            100                 105                 110

Leu Leu Asp Leu Ser Ser Asn Thr Leu Arg Ala Leu Gly Arg His Asp
        115                 120                 125

Leu Asp Gly Leu Gly Ala Leu Glu Lys Leu Leu Leu Phe Asn Asn Arg
```

130 135 140

Leu Val His Leu Asp Glu His Ala Phe His Gly Leu Arg Ala Leu Ser
145 150 155 160

His Leu Tyr Leu Gly Cys Asn Glu Leu Ala Ser Phe Ser Phe Asp His
165 170 175

Leu His Gly Leu Ser Ala Thr His Leu Leu Thr Leu Asp Leu Ser Ser
180 185 190

Asn Arg Leu Gly His Ile Ser Val Pro Glu Leu Ala Ala Leu Pro Ala
195 200 205

Phe Leu Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Pro Cys Asp
210 215 220

Cys Arg Leu Tyr His Leu Leu Gln Arg Trp His Gln Arg Gly Leu Ser
225 230 235 240

Ala Val Arg Asp Phe Ala Arg Glu Tyr Val Cys Leu Ala Phe Lys Val
245 250 255

Pro Ala Ser Arg Val Arg Phe Phe Gln His Ser Arg Val Phe Glu Asn
260 265 270

Cys Ser Ser Ala Pro Ala Leu Gly Leu Glu Arg Pro Glu Glu His Leu
275 280 285

Tyr Ala Leu Val Gly Arg Ser Leu Arg Leu Tyr Cys Asn Thr Ser Val
290 295 300

Pro Ala Met Arg Ile Ala Trp Val Ser Pro Gln Gln Glu Leu Leu Arg
305 310 315 320

Ala Pro Gly Ser Arg Asp Gly Ser Ile Ala Val Leu Ala Asp Gly Ser
325 330 335

Leu Ala Ile Gly Asn Val Gln Glu Gln His Ala Gly Leu Phe Val Cys
340 345 350

Leu Ala Thr Gly Pro Arg Leu His His Asn Gln Thr His Glu Tyr Asn
355 360 365

Val Ser Val His Phe Pro Arg Pro Glu Pro Glu Ala Phe Asn Thr Gly
370 375 380

Phe Thr Thr Leu Leu Gly Cys Ala Val Gly Leu Val Leu Val Leu Leu
385 390 395 400

Tyr Leu Phe Ala Pro Pro Cys Arg Cys Cys Arg Arg Ala Cys Arg Cys
405 410 415

Arg Arg Trp Pro Gln Thr Pro Ser Pro Leu Gln Glu Leu Ser Ala Gln
420 425 430

Ser Ser Val Leu Ser Thr Thr Pro Pro Asp Ala Pro Ser Arg Lys Ala
435 440 445

Ser Val His Lys His Val Val Phe Leu Glu Pro Gly Arg Arg Gly Leu
450 455 460

```
Asn Gly Arg Val Gln Leu Ala Val Ala Glu Glu Phe Asp Leu Tyr Asn
465                 470                 475                 480

Pro Gly Gly Leu Gln Leu Lys Ala Gly Ser Glu Ser Ala Ser Ser Ile
                485                 490                 495

Gly Ser Glu Gly Pro Met Thr Thr
            500
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<210> 7
<211> 1827
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Ig-fusion protein

<220>
<221> CDS
<222> (1)..(1827)

<400> 7

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atg caa ccc cag cgt gac ctg cga ggc ctc tgg ctc ctg ctg ctc tcc   48
Met Gln Pro Gln Arg Asp Leu Arg Gly Leu Trp Leu Leu Leu Leu Ser
  1               5                  10                  15

gtg ttc ctg ctt ctc ttt gag gta gcc agg gcc ggc cga tct gtg gtt   96
Val Phe Leu Leu Leu Phe Glu Val Ala Arg Ala Gly Arg Ser Val Val
             20                  25                  30

agt tgt ccc gcc aac tgc ctg tgc gcc agc aac atc ctc agc tgc tcc  144
Ser Cys Pro Ala Asn Cys Leu Cys Ala Ser Asn Ile Leu Ser Cys Ser
         35                  40                  45

aag cag cag ctg ccc aat gtg ccc caa tct ttg ccc agc tac aca gca  192
Lys Gln Gln Leu Pro Asn Val Pro Gln Ser Leu Pro Ser Tyr Thr Ala
     50                  55                  60

ctg ctg gac ctc agc cac aac aac ttg agc agg ctg cgg gcc gag tgg  240
Leu Leu Asp Leu Ser His Asn Asn Leu Ser Arg Leu Arg Ala Glu Trp
 65                  70                  75                  80

acc ccc acc cgg ctg acc aac ctg cac tcc ctg ctg ctg agc cac aac  288
Thr Pro Thr Arg Leu Thr Asn Leu His Ser Leu Leu Leu Ser His Asn
                 85                  90                  95

cac ctg aac ttc atc tcc tcc gag gcc ttc gtc ccc gta ccc aac ctt  336
His Leu Asn Phe Ile Ser Ser Glu Ala Phe Val Pro Val Pro Asn Leu
            100                 105                 110

agg tac ttg gac ctc tcc tcc aac cat ctt cac acg ctg gat gag ttc  384
Arg Tyr Leu Asp Leu Ser Ser Asn His Leu His Thr Leu Asp Glu Phe
        115                 120                 125

ctg ttc agc gac ctg cag gcg ctg gaa gtg ctg ttg ctc tac aat aac  432
Leu Phe Ser Asp Leu Gln Ala Leu Glu Val Leu Leu Leu Tyr Asn Asn
    130                 135                 140
```

```
cac att gtg gtg gtg gac cgg aat gcc ttt gag gac atg gcc cag ctg   480
His Ile Val Val Val Asp Arg Asn Ala Phe Glu Asp Met Ala Gln Leu
145                 150                 155                 160

cag aaa ctc tac tta agc cag aat cag atc tct cgc ttt cct gtg gaa   528
Gln Lys Leu Tyr Leu Ser Gln Asn Gln Ile Ser Arg Phe Pro Val Glu
                165                 170                 175

ctg atc aag gat ggg aac aaa tta ccc aaa ctg atg ctc ttg gat ctg   576
Leu Ile Lys Asp Gly Asn Lys Leu Pro Lys Leu Met Leu Leu Asp Leu
            180                 185                 190

tcc tcc aac aag ctg aag aag ttg ccc ctg act gac ctg cag aaa ttg   624
Ser Ser Asn Lys Leu Lys Lys Leu Pro Leu Thr Asp Leu Gln Lys Leu
        195                 200                 205

cca gcc tgg gtc aag aat ggg cta tac ctg cat aac aac ccc ttg gag   672
Pro Ala Trp Val Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Glu
    210                 215                 220

tgc gac tgc aag ctc tac cag ctc ttt tcg cac tgg cag tac cgg cag   720
Cys Asp Cys Lys Leu Tyr Gln Leu Phe Ser His Trp Gln Tyr Arg Gln
225                 230                 235                 240

ctg agc tct gtg atg gac ttc cag gag gac ctg tac tgc atg cac tcc   768
Leu Ser Ser Val Met Asp Phe Gln Glu Asp Leu Tyr Cys Met His Ser
                245                 250                 255

aag aag ctg cac aac atc ttc agc ctg gat ttc ttc aat tgc agc gag   816
Lys Lys Leu His Asn Ile Phe Ser Leu Asp Phe Phe Asn Cys Ser Glu
            260                 265                 270

tac aag gaa agt gcc tgg gag gct cac ctg gga gac acc ttg acc atc   864
Tyr Lys Glu Ser Ala Trp Glu Ala His Leu Gly Asp Thr Leu Thr Ile
        275                 280                 285

agg tgt gac acc aaa cag caa ggc atg acc aaa gtg tgg gtg tcc cca   912
Arg Cys Asp Thr Lys Gln Gln Gly Met Thr Lys Val Trp Val Ser Pro
    290                 295                 300

agc aat gaa cag gtg cta agt cag ggg tcc aat ggc tcg gtg agc gtg   960
Ser Asn Glu Gln Val Leu Ser Gln Gly Ser Asn Gly Ser Val Ser Val
305                 310                 315                 320

agg aat ggc gac ctt ttt ttt aaa aag gtg cag gtc gag gat ggg ggt  1008
Arg Asn Gly Asp Leu Phe Phe Lys Lys Val Gln Val Glu Asp Gly Gly
                325                 330                 335

gtg tat acc tgt tac gcc atg ggg gag act ttc aac gag aca ctg tct  1056
Val Tyr Thr Cys Tyr Ala Met Gly Glu Thr Phe Asn Glu Thr Leu Ser
            340                 345                 350

gtg gag ttg aaa gtg tat aac ttc acc ttg cac gga cac cat gac acc  1104
Val Glu Leu Lys Val Tyr Asn Phe Thr Leu His Gly His His Asp Thr
        355                 360                 365

ctc aac gga tcc gag gtg ctg ttc cag ggc ccc aaa tct tgt gac aaa  1152
Leu Asn Gly Ser Glu Val Leu Phe Gln Gly Pro Lys Ser Cys Asp Lys
    370                 375                 380
```

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act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg   1200
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
385                 390                 395                 400

tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc   1248
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                405                 410                 415

cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac   1296
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            420                 425                 430

cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat   1344
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        435                 440                 445

gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg   1392
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    450                 455                 460

gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag   1440
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
465                 470                 475                 480

tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa   1488
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                485                 490                 495

acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc   1536
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            500                 505                 510

ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc   1584
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        515                 520                 525

tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag   1632
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    530                 535                 540

agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg   1680
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
545                 550                 555                 560

gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag   1728
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                565                 570                 575

agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag   1776
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            580                 585                 590

gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt   1824
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        595                 600                 605

aaa                                                               1827
Lys
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<210> 8

<211> 609
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Ig-fusion protein

<400> 8

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Met Gln Pro Gln Arg Asp Leu Arg Gly Leu Trp Leu Leu Leu Leu Ser
 1               5                  10                  15

Val Phe Leu Leu Leu Phe Glu Val Ala Arg Ala Gly Arg Ser Val Val
            20                  25                  30

Ser Cys Pro Ala Asn Cys Leu Cys Ala Ser Asn Ile Leu Ser Cys Ser
        35                  40                  45

Lys Gln Gln Leu Pro Asn Val Pro Gln Ser Leu Pro Ser Tyr Thr Ala
    50                  55                  60

Leu Leu Asp Leu Ser His Asn Asn Leu Ser Arg Leu Arg Ala Glu Trp
65                  70                  75                  80

Thr Pro Thr Arg Leu Thr Asn Leu His Ser Leu Leu Leu Ser His Asn
                85                  90                  95

His Leu Asn Phe Ile Ser Ser Glu Ala Phe Val Pro Val Pro Asn Leu
            100                 105                 110

Arg Tyr Leu Asp Leu Ser Ser Asn His Leu His Thr Leu Asp Glu Phe
        115                 120                 125

Leu Phe Ser Asp Leu Gln Ala Leu Glu Val Leu Leu Leu Tyr Asn Asn
    130                 135                 140

His Ile Val Val Val Asp Arg Asn Ala Phe Glu Asp Met Ala Gln Leu
145                 150                 155                 160

Gln Lys Leu Tyr Leu Ser Gln Asn Gln Ile Ser Arg Phe Pro Val Glu
                165                 170                 175

Leu Ile Lys Asp Gly Asn Lys Leu Pro Lys Leu Met Leu Leu Asp Leu
            180                 185                 190

Ser Ser Asn Lys Leu Lys Lys Leu Pro Leu Thr Asp Leu Gln Lys Leu
        195                 200                 205

Pro Ala Trp Val Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Glu
    210                 215                 220

Cys Asp Cys Lys Leu Tyr Gln Leu Phe Ser His Trp Gln Tyr Arg Gln
225                 230                 235                 240

Leu Ser Ser Val Met Asp Phe Gln Glu Asp Leu Tyr Cys Met His Ser
                245                 250                 255

Lys Lys Leu His Asn Ile Phe Ser Leu Asp Phe Phe Asn Cys Ser Glu
            260                 265                 270

Tyr Lys Glu Ser Ala Trp Glu Ala His Leu Gly Asp Thr Leu Thr Ile
        275                 280                 285
```

```
Arg Cys Asp Thr Lys Gln Gln Gly Met Thr Lys Val Trp Val Ser Pro
    290                 295                 300

Ser Asn Glu Gln Val Leu Ser Gln Gly Ser Asn Gly Ser Val Ser Val
305                 310                 315                 320

Arg Asn Gly Asp Leu Phe Phe Lys Lys Val Gln Val Glu Asp Gly Gly
                325                 330                 335

Val Tyr Thr Cys Tyr Ala Met Gly Glu Thr Phe Asn Glu Thr Leu Ser
            340                 345                 350

Val Glu Leu Lys Val Tyr Asn Phe Thr Leu His Gly His His Asp Thr
        355                 360                 365

Leu Asn Gly Ser Glu Val Leu Phe Gln Gly Pro Lys Ser Cys Asp Lys
    370                 375                 380

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
385                 390                 395                 400

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                405                 410                 415

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            420                 425                 430

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        435                 440                 445

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    450                 455                 460

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
465                 470                 475                 480

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                485                 490                 495

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            500                 505                 510

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        515                 520                 525

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    530                 535                 540

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
545                 550                 555                 560

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                565                 570                 575

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            580                 585                 590

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        595                 600                 605

Lys
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<210> 9
<211> 1920
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Ig-fusion protein

<220>
<221> CDS
<222> (1)..(1920)

<400> 9

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atg tcg tta agg ttc cac aca ctg ccc acc ctg cct aga gct gtc aaa   48
Met Ser Leu Arg Phe His Thr Leu Pro Thr Leu Pro Arg Ala Val Lys
  1               5                  10                  15

ccg ggt tgc aga gag ctg ctg tgt ctg ttg gtg atc gca gtg atg gtg   96
Pro Gly Cys Arg Glu Leu Leu Cys Leu Leu Val Ile Ala Val Met Val
             20                  25                  30

agc ccc agc gcc tca gga atg tgc ccc act gct tgc atc tgt gcc acc  144
Ser Pro Ser Ala Ser Gly Met Cys Pro Thr Ala Cys Ile Cys Ala Thr
         35                  40                  45

gac att gtc agc tgc acc aac aaa aac cta tct aag gtg ccc ggg aac  192
Asp Ile Val Ser Cys Thr Asn Lys Asn Leu Ser Lys Val Pro Gly Asn
     50                  55                  60

ctt ttc aga ctg att aaa aga ctg gat ctg agc tat aac aga atc gga  240
Leu Phe Arg Leu Ile Lys Arg Leu Asp Leu Ser Tyr Asn Arg Ile Gly
 65                  70                  75                  80

ctg ttg gat gcc gac tgg atc ccg gtg tcg ttt gtc aag ctg agc acc  288
Leu Leu Asp Ala Asp Trp Ile Pro Val Ser Phe Val Lys Leu Ser Thr
                 85                  90                  95

tta att ctt cgc cac aac aac atc acc agc atc tcc acg ggc agt ttc  336
Leu Ile Leu Arg His Asn Asn Ile Thr Ser Ile Ser Thr Gly Ser Phe
            100                 105                 110

tcc aca acc cca aat tta aag tgt ctg gac tta tca tcc aat agg ctg  384
Ser Thr Thr Pro Asn Leu Lys Cys Leu Asp Leu Ser Ser Asn Arg Leu
        115                 120                 125

aag tcg gta aag agt gcc aca ttc caa gag ctg aag gct ctg gaa gta  432
Lys Ser Val Lys Ser Ala Thr Phe Gln Glu Leu Lys Ala Leu Glu Val
    130                 135                 140

ctg ctg ctg tac aac aac cac att tcc tat ctg gac ccc gca gcg ttc  480
Leu Leu Leu Tyr Asn Asn His Ile Ser Tyr Leu Asp Pro Ala Ala Phe
145                 150                 155                 160

ggg ggg ctt tcc cac ttg cag aaa ctc tat ctg agt ggg aac ttt ctc  528
Gly Gly Leu Ser His Leu Gln Lys Leu Tyr Leu Ser Gly Asn Phe Leu
                165                 170                 175
```

```
aca cag ttc cct atg gat ttg tat act ggg agg ttc aag ctg gct gat   576
Thr Gln Phe Pro Met Asp Leu Tyr Thr Gly Arg Phe Lys Leu Ala Asp
            180                 185                 190

ctg aca ttt tta gat gtt tcc tat aat cga atc cct tcc ata ccg atg   624
Leu Thr Phe Leu Asp Val Ser Tyr Asn Arg Ile Pro Ser Ile Pro Met
        195                 200                 205

cac cat ata aac tta gtg ccg ggg aga cag ctg aga ggc atc tac ctt   672
His His Ile Asn Leu Val Pro Gly Arg Gln Leu Arg Gly Ile Tyr Leu
    210                 215                 220

cac ggg aac cca ttt gta tgt gac tgt tct ctg tac tcg ttg ctg atc   720
His Gly Asn Pro Phe Val Cys Asp Cys Ser Leu Tyr Ser Leu Leu Ile
225                 230                 235                 240

ttt tgg tac cgt agg cac ttt agc tcc gtg atg gat ttt aag aat gac   768
Phe Trp Tyr Arg Arg His Phe Ser Ser Val Met Asp Phe Lys Asn Asp
                245                 250                 255

tat acc tgt cgc ctg tgg tct gac tcc agg cac tcc cac cag ctg cag   816
Tyr Thr Cys Arg Leu Trp Ser Asp Ser Arg His Ser His Gln Leu Gln
            260                 265                 270

ctg ctc cag gag agc ttt ctg aac tgt tct tac agc gtt atc aac ggc   864
Leu Leu Gln Glu Ser Phe Leu Asn Cys Ser Tyr Ser Val Ile Asn Gly
        275                 280                 285

tcc ttc cac gca ctt ggc ttt atc cac gag gct cag gtt ggg gag agg   912
Ser Phe His Ala Leu Gly Phe Ile His Glu Ala Gln Val Gly Glu Arg
    290                 295                 300

gcg atc gtc cac tgt gac agc aag act ggc aat gga aat act gat ttc   960
Ala Ile Val His Cys Asp Ser Lys Thr Gly Asn Gly Asn Thr Asp Phe
305                 310                 315                 320

atc tgg gtc ggt ccc gat aac agg ctg ctg gag cca gat aaa gac atg   1008
Ile Trp Val Gly Pro Asp Asn Arg Leu Leu Glu Pro Asp Lys Asp Met
                325                 330                 335

ggg aac ttt cgt gtg ttt tac aac gga agt ctg gtc ata gag aac cct   1056
Gly Asn Phe Arg Val Phe Tyr Asn Gly Ser Leu Val Ile Glu Asn Pro
            340                 345                 350

ggc ttt gag gac gcc ggg gta tat tct tgt atc gca atg aac agg cag   1104
Gly Phe Glu Asp Ala Gly Val Tyr Ser Cys Ile Ala Met Asn Arg Gln
        355                 360                 365

cgg ctg tta aac gag acg gtg gat atc atg atc aac gtg agc aat ttc   1152
Arg Leu Leu Asn Glu Thr Val Asp Ile Met Ile Asn Val Ser Asn Phe
    370                 375                 380

acc ata aac aga tcc cac gcc cac gag gcg gcg gcc gcg gat ccc atc   1200
Thr Ile Asn Arg Ser His Ala His Glu Ala Ala Ala Ala Asp Pro Ile
385                 390                 395                 400

gaa ggt cgt ggt ggt ggt ggt ggt gat ccc aaa tct tgt gac aaa cct   1248
Glu Gly Arg Gly Gly Gly Gly Gly Asp Pro Lys Ser Cys Asp Lys Pro
                405                 410                 415
```

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cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca   1296
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
            420                 425                 430

gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg   1344
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
        435                 440                 445

acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct   1392
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
    450                 455                 460

gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc   1440
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
465                 470                 475                 480

aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc   1488
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
                485                 490                 495

agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac   1536
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
            500                 505                 510

aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc   1584
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
        515                 520                 525

atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg   1632
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
    530                 535                 540

ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc   1680
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
545                 550                 555                 560

ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc   1728
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                565                 570                 575

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac   1776
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
            580                 585                 590

tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc   1824
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
        595                 600                 605

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct   1872
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
    610                 615                 620

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa   1920
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
625                 630                 635                 640
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<210> 10
<211> 640
<212> PRT
<213> Artificial Sequence

<223> Description of Artificial Sequence: Ig-fusion protein

<400> 10

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Met Ser Leu Arg Phe His Thr Leu Pro Thr Leu Pro Arg Ala Val Lys
 1               5                  10                  15

Pro Gly Cys Arg Glu Leu Leu Cys Leu Leu Val Ile Ala Val Met Val
            20                  25                  30

Ser Pro Ser Ala Ser Gly Met Cys Pro Thr Ala Cys Ile Cys Ala Thr
        35                  40                  45

Asp Ile Val Ser Cys Thr Asn Lys Asn Leu Ser Lys Val Pro Gly Asn
    50                  55                  60

Leu Phe Arg Leu Ile Lys Arg Leu Asp Leu Ser Tyr Asn Arg Ile Gly
65                  70                  75                  80

Leu Leu Asp Ala Asp Trp Ile Pro Val Ser Phe Val Lys Leu Ser Thr
                85                  90                  95

Leu Ile Leu Arg His Asn Asn Ile Thr Ser Ile Ser Thr Gly Ser Phe
            100                 105                 110

Ser Thr Thr Pro Asn Leu Lys Cys Leu Asp Leu Ser Ser Asn Arg Leu
        115                 120                 125

Lys Ser Val Lys Ser Ala Thr Phe Gln Glu Leu Lys Ala Leu Glu Val
    130                 135                 140

Leu Leu Leu Tyr Asn Asn His Ile Ser Tyr Leu Asp Pro Ala Ala Phe
145                 150                 155                 160

Gly Gly Leu Ser His Leu Gln Lys Leu Tyr Leu Ser Gly Asn Phe Leu
                165                 170                 175

Thr Gln Phe Pro Met Asp Leu Tyr Thr Gly Arg Phe Lys Leu Ala Asp
            180                 185                 190

Leu Thr Phe Leu Asp Val Ser Tyr Asn Arg Ile Pro Ser Ile Pro Met
        195                 200                 205

His His Ile Asn Leu Val Pro Gly Arg Gln Leu Arg Gly Ile Tyr Leu
    210                 215                 220

His Gly Asn Pro Phe Val Cys Asp Cys Ser Leu Tyr Ser Leu Leu Ile
225                 230                 235                 240

Phe Trp Tyr Arg Arg His Phe Ser Ser Val Met Asp Phe Lys Asn Asp
                245                 250                 255

Tyr Thr Cys Arg Leu Trp Ser Asp Ser Arg His Ser His Gln Leu Gln
            260                 265                 270

Leu Leu Gln Glu Ser Phe Leu Asn Cys Ser Tyr Ser Val Ile Asn Gly
        275                 280                 285

Ser Phe His Ala Leu Gly Phe Ile His Glu Ala Gln Val Gly Glu Arg
    290                 295                 300
```

Ala Ile Val His Cys Asp Ser Lys Thr Gly Asn Gly Asn Thr Asp Phe
305 310 315 320

Ile Trp Val Gly Pro Asp Asn Arg Leu Leu Glu Pro Asp Lys Asp Met
325 330 335

Gly Asn Phe Arg Val Phe Tyr Asn Gly Ser Leu Val Ile Glu Asn Pro
340 345 350

Gly Phe Glu Asp Ala Gly Val Tyr Ser Cys Ile Ala Met Asn Arg Gln
355 360 365

Arg Leu Leu Asn Glu Thr Val Asp Ile Met Ile Asn Val Ser Asn Phe
370 375 380

Thr Ile Asn Arg Ser His Ala His Glu Ala Ala Ala Ala Asp Pro Ile
385 390 395 400

Glu Gly Arg Gly Gly Gly Gly Gly Asp Pro Lys Ser Cys Asp Lys Pro
405 410 415

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
420 425 430

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
435 440 445

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
450 455 460

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
465 470 475 480

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
485 490 495

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
500 505 510

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
515 520 525

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
530 535 540

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
545 550 555 560

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
565 570 575

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
580 585 590

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
595 600 605

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
610 615 620

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Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
625                 630                 635                 640
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<210> 11
<211> 1887
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Ig-fusion protein

<220>
<221> CDS
<222> (1)..(1887)

<400> 11

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atg gcc tgg cta gtg cta tca ggt ata cta cta tgc atg ttg ggt gct   48
Met Ala Trp Leu Val Leu Ser Gly Ile Leu Leu Cys Met Leu Gly Ala
  1               5                  10                  15

gga ttg ggc act tca gac ttg gag gat gtt ctg cct cct gct ccc cac   96
Gly Leu Gly Thr Ser Asp Leu Glu Asp Val Leu Pro Pro Ala Pro His
             20                  25                  30

aac tgc ccc gat ata tgc atc tgt gct gcc gat gtg ttg agc tgt gcg  144
Asn Cys Pro Asp Ile Cys Ile Cys Ala Ala Asp Val Leu Ser Cys Ala
         35                  40                  45

ggc cgt ggg tta cag gac ttg ccg gta gca ctg cct acc act gct gca  192
Gly Arg Gly Leu Gln Asp Leu Pro Val Ala Leu Pro Thr Thr Ala Ala
     50                  55                  60

gaa ctc gat ttg agc cac aac gca ctc aaa cgc ctg cac ccg ggg tgg  240
Glu Leu Asp Leu Ser His Asn Ala Leu Lys Arg Leu His Pro Gly Trp
 65                  70                  75                  80

tta gcg ccc ctc tcc cgg ctg cgt gcc ttg cac cta ggc tat aat aag  288
Leu Ala Pro Leu Ser Arg Leu Arg Ala Leu His Leu Gly Tyr Asn Lys
                 85                  90                  95

ctg gaa gtc ctg ggc cat ggt gcg ttc acc aat gcc agt ggc ctg agg  336
Leu Glu Val Leu Gly His Gly Ala Phe Thr Asn Ala Ser Gly Leu Arg
            100                 105                 110

aca ctt gac ctg tcc tct aat atg tta agg atg ctc cat acc cat gac  384
Thr Leu Asp Leu Ser Ser Asn Met Leu Arg Met Leu His Thr His Asp
        115                 120                 125

ctg gat ggc ctg gag gag ctg gag aag tta ctt ctg ttc aat aac agc  432
Leu Asp Gly Leu Glu Glu Leu Glu Lys Leu Leu Leu Phe Asn Asn Ser
    130                 135                 140

ctg atg cac ttg gac ctg gat gcc ttc cag ggc ctg cgc atg ctt agc  480
Leu Met His Leu Asp Leu Asp Ala Phe Gln Gly Leu Arg Met Leu Ser
145                 150                 155                 160

cac ctc tat ctc agc tgc aac gag ctc tcc tct ttc tct ttc aac cac  528
His Leu Tyr Leu Ser Cys Asn Glu Leu Ser Ser Phe Ser Phe Asn His
```

```
                165                 170                 175

ttg cac ggt ctg ggg tta acc cgc ctg cgg act ctg gac ctc tcc tcc   576
Leu His Gly Leu Gly Leu Thr Arg Leu Arg Thr Leu Asp Leu Ser Ser
            180                 185                 190

aac tgg ctg aaa cat atc tcc atc cct gag ttg gct gca ctg cca act   624

Asn Trp Leu Lys His Ile Ser Ile Pro Glu Leu Ala Ala Leu Pro Thr
        195                 200                 205

tat ctc aag aac agg ctc tac ctg cac aac aac ccg ctg ccc tgt gac   672
Tyr Leu Lys Asn Arg Leu Tyr Leu His Asn Asn Pro Leu Pro Cys Asp
    210                 215                 220

tgc agc ctc tac cac ctg ctc cgg cgc tgg cac cag cgg ggc ctg agt   720
Cys Ser Leu Tyr His Leu Leu Arg Arg Trp His Gln Arg Gly Leu Ser
225                 230                 235                 240

gcc ctg cat gat ttt gaa cgc gag tac aca tgc ttg gtc ttt aag gtg   768
Ala Leu His Asp Phe Glu Arg Glu Tyr Thr Cys Leu Val Phe Lys Val
                245                 250                 255

tca gag tcc cga gtg cgc ttt ttt gag cac agc cgg gtc ttc aag aac   816
Ser Glu Ser Arg Val Arg Phe Phe Glu His Ser Arg Val Phe Lys Asn
            260                 265                 270

tgc tct gtg gct gca gct cca ggc tta gag ctg cct gaa gag cag ctg   864
Cys Ser Val Ala Ala Ala Pro Gly Leu Glu Leu Pro Glu Glu Gln Leu
        275                 280                 285

cac gcg cag gtg ggc cag tcc ctg agg ctc ttc tgc aac acc agt gtg   912
His Ala Gln Val Gly Gln Ser Leu Arg Leu Phe Cys Asn Thr Ser Val
    290                 295                 300

cct gcc act cgg gtg gcc tgg gtc tcc ccg aag aat gag ctg ctt gtg   960
Pro Ala Thr Arg Val Ala Trp Val Ser Pro Lys Asn Glu Leu Leu Val
305                 310                 315                 320

gcg cca gcc tct cag gat ggt agc atc gct gtg ttg gct gat ggc agc   1008
Ala Pro Ala Ser Gln Asp Gly Ser Ile Ala Val Leu Ala Asp Gly Ser
                325                 330                 335

tta gcc ata ggc agg gtg caa gag cag cac gca ggc gtc ttt gtg tgc   1056
Leu Ala Ile Gly Arg Val Gln Glu Gln His Ala Gly Val Phe Val Cys
            340                 345                 350

ctg gcc agt ggg ccc cgc ctg cac cac aac cag aca ctt gag tac aat   1104
Leu Ala Ser Gly Pro Arg Leu His His Asn Gln Thr Leu Glu Tyr Asn
        355                 360                 365

gtg agt gtg caa aag gct cgc ccc gag cca gag act ttc aac aca gcg   1152
Val Ser Val Gln Lys Ala Arg Pro Glu Pro Glu Thr Phe Asn Thr Ala
    370                 375                 380

gcc gcg gat ccc atc gaa ggt cgt ggt ggt ggt ggt ggt gat ccc aaa   1200
Ala Ala Asp Pro Ile Glu Gly Arg Gly Gly Gly Gly Gly Asp Pro Lys
385                 390                 395                 400

tct tgt gac aaa cct cac aca tgc cca ccg tgc cca gca cct gaa ctc   1248
Ser Cys Asp Lys Pro His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
```

```
                405                 410                 415

ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc   1296
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            420                 425                 430

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg   1344
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        435                 440                 445

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg   1392
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
    450                 455                 460

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc   1440
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
465                 470                 475                 480

acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg   1488
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                485                 490                 495


aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc   1536
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
            500                 505                 510

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca   1584
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        515                 520                 525

cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag   1632
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
    530                 535                 540

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc   1680
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
545                 550                 555                 560

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg   1728
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                565                 570                 575

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc   1776
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            580                 585                 590

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc   1824
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        595                 600                 605

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc   1872
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    610                 615                 620

ctg tct ccg ggt aaa                                              1887
Leu Ser Pro Gly Lys
625
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<210> 12

<211> 629
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Ig-fusion protein

<400> 12

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Met Ala Trp Leu Val Leu Ser Gly Ile Leu Leu Cys Met Leu Gly Ala
  1               5                  10                  15

Gly Leu Gly Thr Ser Asp Leu Glu Asp Val Leu Pro Pro Ala Pro His
             20                  25                  30

Asn Cys Pro Asp Ile Cys Ile Cys Ala Ala Asp Val Leu Ser Cys Ala
         35                  40                  45

Gly Arg Gly Leu Gln Asp Leu Pro Val Ala Leu Pro Thr Thr Ala Ala
     50                  55                  60

Glu Leu Asp Leu Ser His Asn Ala Leu Lys Arg Leu His Pro Gly Trp
 65                  70                  75                  80

Leu Ala Pro Leu Ser Arg Leu Arg Ala Leu His Leu Gly Tyr Asn Lys
                85                  90                  95

Leu Glu Val Leu Gly His Gly Ala Phe Thr Asn Ala Ser Gly Leu Arg
            100                 105                 110

Thr Leu Asp Leu Ser Ser Asn Met Leu Arg Met Leu His Thr His Asp
        115                 120                 125

Leu Asp Gly Leu Glu Glu Leu Glu Lys Leu Leu Leu Phe Asn Asn Ser
    130                 135                 140

Leu Met His Leu Asp Leu Asp Ala Phe Gln Gly Leu Arg Met Leu Ser
145                 150                 155                 160

His Leu Tyr Leu Ser Cys Asn Glu Leu Ser Ser Phe Ser Phe Asn His
                165                 170                 175

Leu His Gly Leu Gly Leu Thr Arg Leu Arg Thr Leu Asp Leu Ser Ser
            180                 185                 190

Asn Trp Leu Lys His Ile Ser Ile Pro Glu Leu Ala Ala Leu Pro Thr
        195                 200                 205

Tyr Leu Lys Asn Arg Leu Tyr Leu His Asn Asn Pro Leu Pro Cys Asp
    210                 215                 220

Cys Ser Leu Tyr His Leu Leu Arg Arg Trp His Gln Arg Gly Leu Ser
225                 230                 235                 240

Ala Leu His Asp Phe Glu Arg Glu Tyr Thr Cys Leu Val Phe Lys Val
                245                 250                 255

Ser Glu Ser Arg Val Arg Phe Phe Glu His Ser Arg Val Phe Lys Asn
            260                 265                 270

Cys Ser Val Ala Ala Ala Pro Gly Leu Glu Leu Pro Glu Glu Gln Leu
        275                 280                 285
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His Ala Gln Val Gly Gln Ser Leu Arg Leu Phe Cys Asn Thr Ser Val
290 295 300

Pro Ala Thr Arg Val Ala Trp Val Ser Pro Lys Asn Glu Leu Leu Val
305 310 315 320

Ala Pro Ala Ser Gln Asp Gly Ser Ile Ala Val Leu Ala Asp Gly Ser
325 330 335

Leu Ala Ile Gly Arg Val Gln Glu Gln His Ala Gly Val Phe Val Cys
340 345 350

Leu Ala Ser Gly Pro Arg Leu His His Asn Gln Thr Leu Glu Tyr Asn
355 360 365

Val Ser Val Gln Lys Ala Arg Pro Glu Pro Glu Thr Phe Asn Thr Ala
370 375 380

Ala Ala Asp Pro Ile Glu Gly Arg Gly Gly Gly Gly Gly Asp Pro Lys
385 390 395 400

Ser Cys Asp Lys Pro His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
405 410 415

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
420 425 430

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
435 440 445

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
450 455 460

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
465 470 475 480

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
485 490 495

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
500 505 510

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
515 520 525

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
530 535 540

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
545 550 555 560

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
565 570 575

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
580 585 590

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
595 600 605

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser

610 615 620

Leu Ser Pro Gly Lys
625

<210> 13
<211> 1476
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(1476)

<400> 13

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atg caa ccc cag cgt gac ctg cga ggc ctc tgg ctc ctg ctg ctc tcc   48
Met Gln Pro Gln Arg Asp Leu Arg Gly Leu Trp Leu Leu Leu Leu Ser
  1               5                  10                  15

gtg ttc ctg ctt ctc ttt gag gta gcc agg gcc ggc cga tct gtg gtt   96
Val Phe Leu Leu Leu Phe Glu Val Ala Arg Ala Gly Arg Ser Val Val
             20                  25                  30

agt tgt ccc gcc aac tgc ctg tgc gcc agc aac atc ctc agc tgc tcc  144
Ser Cys Pro Ala Asn Cys Leu Cys Ala Ser Asn Ile Leu Ser Cys Ser
         35                  40                  45

aag cag cag ctg ccc aat gtg ccc caa tct ttg ccc agc tac aca gca  192
Lys Gln Gln Leu Pro Asn Val Pro Gln Ser Leu Pro Ser Tyr Thr Ala
     50                  55                  60

ctg ctg gac ctc agc cac aac aac ttg agc agg ctg cgg gcc gag tgg  240
Leu Leu Asp Leu Ser His Asn Asn Leu Ser Arg Leu Arg Ala Glu Trp
 65                  70                  75                  80

acc ccc acc cgg ctg acc aac ctg cac tcc ctg ctg ctg agc cac aac  288
Thr Pro Thr Arg Leu Thr Asn Leu His Ser Leu Leu Leu Ser His Asn
                 85                  90                  95

cac ctg aac ttc atc tcc tcc gag gcc ttc gtc ccc gta ccc aac ctt  336
His Leu Asn Phe Ile Ser Ser Glu Ala Phe Val Pro Val Pro Asn Leu
            100                 105                 110

agg tac ttg gac ctc tcc tcc aac cat ctt cac acg ctg gat gag ttc  384
Arg Tyr Leu Asp Leu Ser Ser Asn His Leu His Thr Leu Asp Glu Phe
        115                 120                 125

ctg ttc agc gac ctg cag gcg ctg gaa gtg ctg ttg ctc tac aat aac  432
Leu Phe Ser Asp Leu Gln Ala Leu Glu Val Leu Leu Leu Tyr Asn Asn
    130                 135                 140

cac att gtg gtg gtg gac cgg aat gcc ttt gag gac atg gcc cag ctg  480
His Ile Val Val Val Asp Arg Asn Ala Phe Glu Asp Met Ala Gln Leu
145                 150                 155                 160

cag aaa ctc tac tta agc cag aat cag atc tct cgc ttt cct gtg gaa  528
Gln Lys Leu Tyr Leu Ser Gln Asn Gln Ile Ser Arg Phe Pro Val Glu
                165                 170                 175
```

```
ctg atc aag gat ggg aac aaa tta ccc aaa ctg atg ctc ttg gat ctg   576
Leu Ile Lys Asp Gly Asn Lys Leu Pro Lys Leu Met Leu Leu Asp Leu
            180                 185                 190

tcc tcc aac aag ctg aag aag ttg ccc ctg act gac ctg cag aaa ttg   624
Ser Ser Asn Lys Leu Lys Lys Leu Pro Leu Thr Asp Leu Gln Lys Leu
        195                 200                 205

cca gcc tgg gtc aag aat ggg cta tac ctg cat aac aac ccc ttg gag   672
Pro Ala Trp Val Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Glu
    210                 215                 220

tgc gac tgc aag ctc tac cag ctc ttt tcg cac tgg cag tac cgg cag   720
Cys Asp Cys Lys Leu Tyr Gln Leu Phe Ser His Trp Gln Tyr Arg Gln
225                 230                 235                 240

ctg agc tct gtg atg gac ttc cag gag gac ctg tac tgc atg cac tcc   768
Leu Ser Ser Val Met Asp Phe Gln Glu Asp Leu Tyr Cys Met His Ser
                245                 250                 255


aag aag ctg cac aac atc ttc agc ctg gat ttc ttc aat tgc agc gag   816
Lys Lys Leu His Asn Ile Phe Ser Leu Asp Phe Phe Asn Cys Ser Glu
            260                 265                 270

tac aag gaa agt gcc tgg gag gct cac ctg gga gac acc ttg acc atc   864
Tyr Lys Glu Ser Ala Trp Glu Ala His Leu Gly Asp Thr Leu Thr Ile
        275                 280                 285

agg tgt gac acc aaa cag caa ggc atg acc aaa gtg tgg gtg tcc cca   912
Arg Cys Asp Thr Lys Gln Gln Gly Met Thr Lys Val Trp Val Ser Pro
    290                 295                 300

agc aat gaa cag gtg cta agt cag ggg tcc aat ggc tcg gtg agc gtg   960
Ser Asn Glu Gln Val Leu Ser Gln Gly Ser Asn Gly Ser Val Ser Val
305                 310                 315                 320

agg aat ggc gac ctt ttt ttt aaa aag gtg cag gtc gag gat ggg ggt  1008
Arg Asn Gly Asp Leu Phe Phe Lys Lys Val Gln Val Glu Asp Gly Gly
                325                 330                 335

gtg tat acc tgt tac gcc atg ggg gag act ttc aac gag aca ctg tct  1056
Val Tyr Thr Cys Tyr Ala Met Gly Glu Thr Phe Asn Glu Thr Leu Ser
            340                 345                 350

gtg gag ttg aaa gtg tat aac ttc acc ttg cac gga cac cat gac acc  1104
Val Glu Leu Lys Val Tyr Asn Phe Thr Leu His Gly His His Asp Thr
        355                 360                 365

ctc aac aca gcc tac act acc ctg gtg ggc tgt atc ctc agt gtg gtt  1152
Leu Asn Thr Ala Tyr Thr Thr Leu Val Gly Cys Ile Leu Ser Val Val
    370                 375                 380

ctg gtc ctc ata tac ttg tac ctc acc cct tgc cgc tgc tgg tgt cgg  1200
Leu Val Leu Ile Tyr Leu Tyr Leu Thr Pro Cys Arg Cys Trp Cys Arg
385                 390                 395                 400

ggt gtg gag aaa cct tcc agc cac caa gga gat agc ctc agc tct tct  1248
Gly Val Glu Lys Pro Ser Ser His Gln Gly Asp Ser Leu Ser Ser Ser
                405                 410                 415
```

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atg ctc agt acc aca ccc aac cac gac cct atg gct ggt ggg gac aaa   1296
Met Leu Ser Thr Thr Pro Asn His Asp Pro Met Ala Gly Gly Asp Lys
            420                 425                 430

gat gat ggt ttt gac cgg cgg gtg gcc ttc ctg gaa cct gct gga ccc   1344
Asp Asp Gly Phe Asp Arg Arg Val Ala Phe Leu Glu Pro Ala Gly Pro
        435                 440                 445

ggg cag ggt caa aat ggc aaa ctc aag cca ggc aac act ctg ccg gtg   1392
Gly Gln Gly Gln Asn Gly Lys Leu Lys Pro Gly Asn Thr Leu Pro Val
    450                 455                 460

ccc gaa gct aca ggc aag ggc caa cgg agg atg tcc gat cca gag tcg   1440
Pro Glu Ala Thr Gly Lys Gly Gln Arg Arg Met Ser Asp Pro Glu Ser
465                 470                 475                 480

gtc agc tcg gtc ttt tct gat aca ccc att gtg gtg                   1476
Val Ser Ser Val Phe Ser Asp Thr Pro Ile Val Val
                485                 490
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<210> 14
<211> 492
<212> PRT
<213> Mus musculus

<400> 14

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Met Gln Pro Gln Arg Asp Leu Arg Gly Leu Trp Leu Leu Leu Leu Ser
  1               5                  10                  15

Val Phe Leu Leu Leu Phe Glu Val Ala Arg Ala Gly Arg Ser Val Val
            20                  25                  30

Ser Cys Pro Ala Asn Cys Leu Cys Ala Ser Asn Ile Leu Ser Cys Ser
        35                  40                  45

Lys Gln Gln Leu Pro Asn Val Pro Gln Ser Leu Pro Ser Tyr Thr Ala
    50                  55                  60

Leu Leu Asp Leu Ser His Asn Asn Leu Ser Arg Leu Arg Ala Glu Trp
65                  70                  75                  80

Thr Pro Thr Arg Leu Thr Asn Leu His Ser Leu Leu Leu Ser His Asn
                85                  90                  95

His Leu Asn Phe Ile Ser Ser Glu Ala Phe Val Pro Val Pro Asn Leu
            100                 105                 110

Arg Tyr Leu Asp Leu Ser Ser Asn His Leu His Thr Leu Asp Glu Phe
        115                 120                 125

Leu Phe Ser Asp Leu Gln Ala Leu Glu Val Leu Leu Leu Tyr Asn Asn
    130                 135                 140

His Ile Val Val Val Asp Arg Asn Ala Phe Glu Asp Met Ala Gln Leu
145                 150                 155                 160

Gln Lys Leu Tyr Leu Ser Gln Asn Gln Ile Ser Arg Phe Pro Val Glu
                165                 170                 175

Leu Ile Lys Asp Gly Asn Lys Leu Pro Lys Leu Met Leu Leu Asp Leu
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180 185 190

Ser Ser Asn Lys Leu Lys Lys Leu Pro Leu Thr Asp Leu Gln Lys Leu
195 200 205

Pro Ala Trp Val Lys Asn Gly Leu Tyr Leu His Asn Asn Pro Leu Glu
210 215 220

Cys Asp Cys Lys Leu Tyr Gln Leu Phe Ser His Trp Gln Tyr Arg Gln
225 230 235 240

Leu Ser Ser Val Met Asp Phe Gln Glu Asp Leu Tyr Cys Met His Ser
245 250 255

Lys Lys Leu His Asn Ile Phe Ser Leu Asp Phe Phe Asn Cys Ser Glu
260 265 270

Tyr Lys Glu Ser Ala Trp Glu Ala His Leu Gly Asp Thr Leu Thr Ile
275 280 285

Arg Cys Asp Thr Lys Gln Gln Gly Met Thr Lys Val Trp Val Ser Pro
290 295 300

Ser Asn Glu Gln Val Leu Ser Gln Gly Ser Asn Gly Ser Val Ser Val
305 310 315 320

Arg Asn Gly Asp Leu Phe Phe Lys Lys Val Gln Val Glu Asp Gly Gly
325 330 335

Val Tyr Thr Cys Tyr Ala Met Gly Glu Thr Phe Asn Glu Thr Leu Ser
340 345 350

Val Glu Leu Lys Val Tyr Asn Phe Thr Leu His Gly His His Asp Thr
355 360 365

Leu Asn Thr Ala Tyr Thr Thr Leu Val Gly Cys Ile Leu Ser Val Val
370 375 380

Leu Val Leu Ile Tyr Leu Tyr Leu Thr Pro Cys Arg Cys Trp Cys Arg
385 390 395 400

Gly Val Glu Lys Pro Ser Ser His Gln Gly Asp Ser Leu Ser Ser Ser
405 410 415

Met Leu Ser Thr Thr Pro Asn His Asp Pro Met Ala Gly Gly Asp Lys
420 425 430

Asp Asp Gly Phe Asp Arg Arg Val Ala Phe Leu Glu Pro Ala Gly Pro
435 440 445

Gly Gln Gly Gln Asn Gly Lys Leu Lys Pro Gly Asn Thr Leu Pro Val
450 455 460

Pro Glu Ala Thr Gly Lys Gly Gln Arg Arg Met Ser Asp Pro Glu Ser
465 470 475 480

Val Ser Ser Val Phe Ser Asp Thr Pro Ile Val Val
485 490

<210> 15
<211> 1557
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(1557)

<400> 15
atg tcg tta agg ttc cac aca ctg ccc acc ctg cct aga gct gtc aaa 48
Met Ser Leu Arg Phe His Thr Leu Pro Thr Leu Pro Arg Ala Val Lys
1 5 10 15

ccg ggt tgc aga gag ctg ctg tgt ctg ttg gtg atc gca gtg atg gtg 96
Pro Gly Cys Arg Glu Leu Leu Cys Leu Leu Val Ile Ala Val Met Val
20 25 30

agc ccc agc gcc tca gga atg tgc ccc act gct tgc atc tgt gcc acc 144
Ser Pro Ser Ala Ser Gly Met Cys Pro Thr Ala Cys Ile Cys Ala Thr
35 40 45

gac att gtc agc tgc acc aac aaa aac cta tct aag gtg ccc ggg aac 192
Asp Ile Val Ser Cys Thr Asn Lys Asn Leu Ser Lys Val Pro Gly Asn
50 55 60

ctt ttc aga ctg att aaa aga ctg gat ctg agc tat aac aga atc gga 240
Leu Phe Arg Leu Ile Lys Arg Leu Asp Leu Ser Tyr Asn Arg Ile Gly
65 70 75 80

ctg ttg gat gcc gac tgg atc ccg gtg tcg ttt gtc aag ctg agc acc 288
Leu Leu Asp Ala Asp Trp Ile Pro Val Ser Phe Val Lys Leu Ser Thr
85 90 95

tta att ctt cgc cac aac aac atc acc agc atc tcc acg ggc agt ttc 336
Leu Ile Leu Arg His Asn Asn Ile Thr Ser Ile Ser Thr Gly Ser Phe
100 105 110

tcc aca acc cca aat tta aag tgt ctg gac tta tca tcc aat agg ctg 384
Ser Thr Thr Pro Asn Leu Lys Cys Leu Asp Leu Ser Ser Asn Arg Leu
115 120 125

aag tcg gta aag agt gcc aca ttc caa gag ctg aag gct ctg gaa gta 432
Lys Ser Val Lys Ser Ala Thr Phe Gln Glu Leu Lys Ala Leu Glu Val
130 135 140

ctg ctg ctg tac aac aac cac att tcc tat ctg gac ccc gca gcg ttc 480
Leu Leu Leu Tyr Asn Asn His Ile Ser Tyr Leu Asp Pro Ala Ala Phe
145 150 155 160

ggg ggg ctt tcc cac ttg cag aaa ctc tat ctg agt ggg aac ttt ctc 528
Gly Gly Leu Ser His Leu Gln Lys Leu Tyr Leu Ser Gly Asn Phe Leu
165 170 175

aca cag ttc cct atg gat ttg tat act ggg agg ttc aag ctg gct gat 576
Thr Gln Phe Pro Met Asp Leu Tyr Thr Gly Arg Phe Lys Leu Ala Asp
180 185 190

ctg aca ttt tta gat gtt tcc tat aat cga atc cct tcc ata ccg atg 624
Leu Thr Phe Leu Asp Val Ser Tyr Asn Arg Ile Pro Ser Ile Pro Met
195 200 205

```
cac cat ata aac tta gtg ccg ggg aga cag ctg aga ggc atc tac ctt   672
His His Ile Asn Leu Val Pro Gly Arg Gln Leu Arg Gly Ile Tyr Leu
    210                 215                 220

cac ggg aac cca ttt gta tgt gac tgt tct ctg tac tcg ttg ctg atc   720
His Gly Asn Pro Phe Val Cys Asp Cys Ser Leu Tyr Ser Leu Leu Ile
225                 230                 235                 240

ttt tgg tac cgt agg cac ttt agc tcc gtg atg gat ttt aag aat gac   768
Phe Trp Tyr Arg Arg His Phe Ser Ser Val Met Asp Phe Lys Asn Asp
                245                 250                 255

tat acc tgt cgc ctg tgg tct gac tcc agg cac tcc cac cag ctg cag   816
Tyr Thr Cys Arg Leu Trp Ser Asp Ser Arg His Ser His Gln Leu Gln
            260                 265                 270

ctg ctc cag gag agc ttt ctg aac tgt tct tac agc gtt atc aac ggc   864
Leu Leu Gln Glu Ser Phe Leu Asn Cys Ser Tyr Ser Val Ile Asn Gly
        275                 280                 285

tcc ttc cac gca ctt ggc ttt atc cac gag gct cag gtt ggg gag agg   912
Ser Phe His Ala Leu Gly Phe Ile His Glu Ala Gln Val Gly Glu Arg
    290                 295                 300

gcg atc gtc cac tgt gac agc aag act ggc aat gga aat act gat ttc   960
Ala Ile Val His Cys Asp Ser Lys Thr Gly Asn Gly Asn Thr Asp Phe
305                 310                 315                 320

atc tgg gtc ggt ccc gat aac agg ctg ctg gag cca gat aaa gac atg   1008
Ile Trp Val Gly Pro Asp Asn Arg Leu Leu Glu Pro Asp Lys Asp Met
                325                 330                 335

ggg aac ttt cgt gtg ttt tac aac gga agt ctg gtc ata gag aac cct   1056
Gly Asn Phe Arg Val Phe Tyr Asn Gly Ser Leu Val Ile Glu Asn Pro
            340                 345                 350

ggc ttt gag gac gcc ggg gta tat tct tgt atc gca atg aac agg cag   1104
Gly Phe Glu Asp Ala Gly Val Tyr Ser Cys Ile Ala Met Asn Arg Gln
        355                 360                 365

cgg ctg tta aac gag acg gtg gat atc atg atc aac gtg agc aat ttc   1152
Arg Leu Leu Asn Glu Thr Val Asp Ile Met Ile Asn Val Ser Asn Phe
    370                 375                 380

acc ata aac aga tcc cac gcc cac gag gcg ttt aac acg gcc ttt acc   1200
Thr Ile Asn Arg Ser His Ala His Glu Ala Phe Asn Thr Ala Phe Thr
385                 390                 395                 400

acc ctg gct gcc tgc gtg gcc agt ata gtt cta gtg cta ctg tat ctg   1248
Thr Leu Ala Ala Cys Val Ala Ser Ile Val Leu Val Leu Leu Tyr Leu
                405                 410                 415

tac ctg acg ccg tgc cca tgc aaa tgc aaa gcc aag aga cag aaa aac   1296
Tyr Leu Thr Pro Cys Pro Cys Lys Cys Lys Ala Lys Arg Gln Lys Asn
            420                 425                 430

acg ctg agc caa agc agt gcc cac tcg tcc att ctc agt cct ggc ccc   1344
Thr Leu Ser Gln Ser Ser Ala His Ser Ser Ile Leu Ser Pro Gly Pro
        435                 440                 445
```

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act ggc gat gcc tct gct gac gat cgg aag gca ggt aaa aga gtc gtg   1392
Thr Gly Asp Ala Ser Ala Asp Asp Arg Lys Ala Gly Lys Arg Val Val
    450                 455                 460

ttt ctg gag ccc ctg aag gac acg gcg gcc gga cag aat ggc aaa gtc   1440
Phe Leu Glu Pro Leu Lys Asp Thr Ala Ala Gly Gln Asn Gly Lys Val
465                 470                 475                 480

aag ctt ttc ccc agt gag acc gtt ata gcc gag ggc atc tta aag tcc   1488
Lys Leu Phe Pro Ser Glu Thr Val Ile Ala Glu Gly Ile Leu Lys Ser
                485                 490                 495

acc agg gca aag tct gac tca gac tca gtc aat tcc gtg ttc tca gac   1536
Thr Arg Ala Lys Ser Asp Ser Asp Ser Val Asn Ser Val Phe Ser Asp
            500                 505                 510

aca ccc ttt gtg gca tcc act                                     1557
Thr Pro Phe Val Ala Ser Thr
        515
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<210> 16
<211> 519
<212> PRT
<213> Mus musculus

<400> 16

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Met Ser Leu Arg Phe His Thr Leu Pro Thr Leu Pro Arg Ala Val Lys
  1               5                  10                  15

Pro Gly Cys Arg Glu Leu Leu Cys Leu Leu Val Ile Ala Val Met Val
            20                  25                  30

Ser Pro Ser Ala Ser Gly Met Cys Pro Thr Ala Cys Ile Cys Ala Thr
        35                  40                  45

Asp Ile Val Ser Cys Thr Asn Lys Asn Leu Ser Lys Val Pro Gly Asn
    50                  55                  60

Leu Phe Arg Leu Ile Lys Arg Leu Asp Leu Ser Tyr Asn Arg Ile Gly
65                  70                  75                  80

Leu Leu Asp Ala Asp Trp Ile Pro Val Ser Phe Val Lys Leu Ser Thr
                85                  90                  95

Leu Ile Leu Arg His Asn Asn Ile Thr Ser Ile Ser Thr Gly Ser Phe
            100                 105                 110

Ser Thr Thr Pro Asn Leu Lys Cys Leu Asp Leu Ser Ser Asn Arg Leu
        115                 120                 125

Lys Ser Val Lys Ser Ala Thr Phe Gln Glu Leu Lys Ala Leu Glu Val
    130                 135                 140

Leu Leu Leu Tyr Asn Asn His Ile Ser Tyr Leu Asp Pro Ala Ala Phe
145                 150                 155                 160

Gly Gly Leu Ser His Leu Gln Lys Leu Tyr Leu Ser Gly Asn Phe Leu
                165                 170                 175

Thr Gln Phe Pro Met Asp Leu Tyr Thr Gly Arg Phe Lys Leu Ala Asp
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```
          180                 185                 190

Leu Thr Phe Leu Asp Val Ser Tyr Asn Arg Ile Pro Ser Ile Pro Met
        195                 200                 205

His His Ile Asn Leu Val Pro Gly Arg Gln Leu Arg Gly Ile Tyr Leu
    210                 215                 220

His Gly Asn Pro Phe Val Cys Asp Cys Ser Leu Tyr Ser Leu Leu Ile
225                 230                 235                 240

Phe Trp Tyr Arg Arg His Phe Ser Ser Val Met Asp Phe Lys Asn Asp
                245                 250                 255

Tyr Thr Cys Arg Leu Trp Ser Asp Ser Arg His Ser His Gln Leu Gln
            260                 265                 270

Leu Leu Gln Glu Ser Phe Leu Asn Cys Ser Tyr Ser Val Ile Asn Gly
        275                 280                 285

Ser Phe His Ala Leu Gly Phe Ile His Glu Ala Gln Val Gly Glu Arg
    290                 295                 300

Ala Ile Val His Cys Asp Ser Lys Thr Gly Asn Gly Asn Thr Asp Phe
305                 310                 315                 320

Ile Trp Val Gly Pro Asp Asn Arg Leu Leu Glu Pro Asp Lys Asp Met
                325                 330                 335

Gly Asn Phe Arg Val Phe Tyr Asn Gly Ser Leu Val Ile Glu Asn Pro
            340                 345                 350

Gly Phe Glu Asp Ala Gly Val Tyr Ser Cys Ile Ala Met Asn Arg Gln
        355                 360                 365

Arg Leu Leu Asn Glu Thr Val Asp Ile Met Ile Asn Val Ser Asn Phe
    370                 375                 380

Thr Ile Asn Arg Ser His Ala His Glu Ala Phe Asn Thr Ala Phe Thr
385                 390                 395                 400

Thr Leu Ala Ala Cys Val Ala Ser Ile Val Leu Val Leu Leu Tyr Leu
                405                 410                 415

Tyr Leu Thr Pro Cys Pro Cys Lys Cys Lys Ala Lys Arg Gln Lys Asn
            420                 425                 430

Thr Leu Ser Gln Ser Ser Ala His Ser Ser Ile Leu Ser Pro Gly Pro
        435                 440                 445

Thr Gly Asp Ala Ser Ala Asp Asp Arg Lys Ala Gly Lys Arg Val Val
    450                 455                 460

Phe Leu Glu Pro Leu Lys Asp Thr Ala Ala Gly Gln Asn Gly Lys Val
465                 470                 475                 480

Lys Leu Phe Pro Ser Glu Thr Val Ile Ala Glu Gly Ile Leu Lys Ser
                485                 490                 495

Thr Arg Ala Lys Ser Asp Ser Asp Ser Val Asn Ser Val Phe Ser Asp
            500                 505                 510
```

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Thr Pro Phe Val Ala Ser Thr
        515
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<210> 17
<211> 1524
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(1524)

<400> 17

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atg gcc tgg cta gtg cta tca ggt ata cta cta tgc atg ttg ggt gct   48
Met Ala Trp Leu Val Leu Ser Gly Ile Leu Leu Cys Met Leu Gly Ala
  1               5                  10                  15

gga ttg ggc act tca gac ttg gag gat gtt ctg cct cct gct ccc cac   96
Gly Leu Gly Thr Ser Asp Leu Glu Asp Val Leu Pro Pro Ala Pro His
            20                  25                  30

aac tgc ccc gat ata tgc atc tgt gct gcc gat gtg ttg agc tgt gcg   144
Asn Cys Pro Asp Ile Cys Ile Cys Ala Ala Asp Val Leu Ser Cys Ala
         35                  40                  45


ggc cgt ggg tta cag gac ttg ccg gta gca ctg cct acc act gct gca   192
Gly Arg Gly Leu Gln Asp Leu Pro Val Ala Leu Pro Thr Thr Ala Ala
     50                  55                  60

gaa ctc gat ttg agc cac aac gca ctc aaa cgc ctg cac ccg ggg tgg   240
Glu Leu Asp Leu Ser His Asn Ala Leu Lys Arg Leu His Pro Gly Trp
 65                  70                  75                  80

tta gcg ccc ctc tcc cgg ctg cgt gcc ttg cac cta ggc tat aat aag   288
Leu Ala Pro Leu Ser Arg Leu Arg Ala Leu His Leu Gly Tyr Asn Lys
                85                  90                  95

ctg gaa gtc ctg ggc cat ggt gcg ttc acc aat gcc agt ggc ctg agg   336
Leu Glu Val Leu Gly His Gly Ala Phe Thr Asn Ala Ser Gly Leu Arg
            100                 105                 110

aca ctt gac ctg tcc tct aat atg tta agg atg ctc cat acc cat gac   384
Thr Leu Asp Leu Ser Ser Asn Met Leu Arg Met Leu His Thr His Asp
        115                 120                 125

ctg gat ggc ctg gag gag ctg gag aag tta ctt ctg ttc aat aac agc   432
Leu Asp Gly Leu Glu Glu Leu Glu Lys Leu Leu Leu Phe Asn Asn Ser
    130                 135                 140

ctg atg cac ttg gac ctg gat gcc ttc cag ggc ctg cgc atg ctt agc   480
Leu Met His Leu Asp Leu Asp Ala Phe Gln Gly Leu Arg Met Leu Ser
145                 150                 155                 160

cac ctc tat ctc agc tgc aac gag ctc tcc tct ttc tct ttc aac cac   528
His Leu Tyr Leu Ser Cys Asn Glu Leu Ser Ser Phe Ser Phe Asn His
                165                 170                 175
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```
ttg cac ggt ctg ggg tta acc cgc ctg cgg act ctg gac ctc tcc tcc   576
Leu His Gly Leu Gly Leu Thr Arg Leu Arg Thr Leu Asp Leu Ser Ser
            180                 185                 190

aac tgg ctg aaa cat atc tcc atc cct gag ttg gct gca ctg cca act   624
Asn Trp Leu Lys His Ile Ser Ile Pro Glu Leu Ala Ala Leu Pro Thr
        195                 200                 205

tat ctc aag aac agg ctc tac ctg cac aac aac ccg ctg ccc tgt gac   672
Tyr Leu Lys Asn Arg Leu Tyr Leu His Asn Asn Pro Leu Pro Cys Asp
    210                 215                 220

tgc agc ctc tac cac ctg ctc cgg cgc tgg cac cag cgg ggc ctg agt   720
Cys Ser Leu Tyr His Leu Leu Arg Arg Trp His Gln Arg Gly Leu Ser
225                 230                 235                 240

gcc ctg cat gat ttt gaa cgc gag tac aca tgc ttg gtc ttt aag gtg   768
Ala Leu His Asp Phe Glu Arg Glu Tyr Thr Cys Leu Val Phe Lys Val
                245                 250                 255

tca gag tcc cga gtg cgc ttt ttt gag cac agc cgg gtc ttc aag aac   816
Ser Glu Ser Arg Val Arg Phe Phe Glu His Ser Arg Val Phe Lys Asn
            260                 265                 270

tgc tct gtg gct gca gct cca ggc tta gag ctg cct gaa gag cag ctg   864
Cys Ser Val Ala Ala Ala Pro Gly Leu Glu Leu Pro Glu Glu Gln Leu
        275                 280                 285

cac gcg cag gtg ggc cag tcc ctg agg ctc ttc tgc aac acc agt gtg   912
His Ala Gln Val Gly Gln Ser Leu Arg Leu Phe Cys Asn Thr Ser Val
    290                 295                 300

cct gcc act cgg gtg gcc tgg gtc tcc ccg aag aat gag ctg ctt gtg   960
Pro Ala Thr Arg Val Ala Trp Val Ser Pro Lys Asn Glu Leu Leu Val
305                 310                 315                 320

gcg cca gcc tct cag gat ggt agc atc gct gtg ttg gct gat ggc agc  1008
Ala Pro Ala Ser Gln Asp Gly Ser Ile Ala Val Leu Ala Asp Gly Ser
                325                 330                 335

tta gcc ata ggc agg gtg caa gag cag cac gca ggc gtc ttt gtg tgc  1056
Leu Ala Ile Gly Arg Val Gln Glu Gln His Ala Gly Val Phe Val Cys
            340                 345                 350

ctg gcc agt ggg ccc cgc ctg cac cac aac cag aca ctt gag tac aat  1104
Leu Ala Ser Gly Pro Arg Leu His His Asn Gln Thr Leu Glu Tyr Asn
        355                 360                 365

gtg agt gtg caa aag gct cgc ccc gag cca gag act ttc aac aca ggc  1152
Val Ser Val Gln Lys Ala Arg Pro Glu Pro Glu Thr Phe Asn Thr Gly
    370                 375                 380

ttt acc acc ctg ctg ggc tgt att gtg ggc ctg gtg ctg gtg ttg ctc  1200
Phe Thr Thr Leu Leu Gly Cys Ile Val Gly Leu Val Leu Val Leu Leu
385                 390                 395                 400

tac ttg ttt gca cca ccc tgt cgt ggc tgc tgt cac tgc tgt cag cgg  1248
Tyr Leu Phe Ala Pro Pro Cys Arg Gly Cys Cys His Cys Cys Gln Arg
                405                 410                 415

gcc tgc cgc aac cgt tgc tgg ccc cgg gca tcc agt cca ctc cag gag  1296
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Ala Cys Arg Asn Arg Cys Trp Pro Arg Ala Ser Ser Pro Leu Gln Glu
            420                 425                 430

ctg agc gca cag tcc tcc atg ctc agc act acg cca cca gat gca ccc   1344
Leu Ser Ala Gln Ser Ser Met Leu Ser Thr Thr Pro Pro Asp Ala Pro
        435                 440                 445

agc cgc aag gcc agt gtc cac aag cat gtg gtc ttc ctg gag ccg ggc   1392
Ser Arg Lys Ala Ser Val His Lys His Val Val Phe Leu Glu Pro Gly
    450                 455                 460

aag aag ggc ctc aat ggc cgt gtg cag ctc gca gta gct gaa gac ttc   1440
Lys Lys Gly Leu Asn Gly Arg Val Gln Leu Ala Val Ala Glu Asp Phe
465                 470                 475                 480

gat ctg tgc aac ccc atg ggc ttg caa ctc aag gct ggc tct gaa tca   1488
Asp Leu Cys Asn Pro Met Gly Leu Gln Leu Lys Ala Gly Ser Glu Ser
                485                 490                 495

gcc agt tcc acg ggc tca gag ggt ctc gtg atg agc                   1524
Ala Ser Ser Thr Gly Ser Glu Gly Leu Val Met Ser
            500                 505


<210> 18
<211> 508
<212> PRT
<213> Mus musculus

<400> 18
Met Ala Trp Leu Val Leu Ser Gly Ile Leu Leu Cys Met Leu Gly Ala
  1               5                  10                  15


Gly Leu Gly Thr Ser Asp Leu Glu Asp Val Leu Pro Pro Ala Pro His
             20                  25                  30

Asn Cys Pro Asp Ile Cys Ile Cys Ala Ala Asp Val Leu Ser Cys Ala
         35                  40                  45

Gly Arg Gly Leu Gln Asp Leu Pro Val Ala Leu Pro Thr Thr Ala Ala
     50                  55                  60

Glu Leu Asp Leu Ser His Asn Ala Leu Lys Arg Leu His Pro Gly Trp
 65                  70                  75                  80

Leu Ala Pro Leu Ser Arg Leu Arg Ala Leu His Leu Gly Tyr Asn Lys
                 85                  90                  95

Leu Glu Val Leu Gly His Gly Ala Phe Thr Asn Ala Ser Gly Leu Arg
            100                 105                 110

Thr Leu Asp Leu Ser Ser Asn Met Leu Arg Met Leu His Thr His Asp
        115                 120                 125

Leu Asp Gly Leu Glu Glu Leu Glu Lys Leu Leu Leu Phe Asn Asn Ser
    130                 135                 140

Leu Met His Leu Asp Leu Asp Ala Phe Gln Gly Leu Arg Met Leu Ser
145                 150                 155                 160
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```
His Leu Tyr Leu Ser Cys Asn Glu Leu Ser Ser Phe Ser Phe Asn His
                165                 170                 175

Leu His Gly Leu Gly Leu Thr Arg Leu Arg Thr Leu Asp Leu Ser Ser
            180                 185                 190

Asn Trp Leu Lys His Ile Ser Ile Pro Glu Leu Ala Ala Leu Pro Thr
        195                 200                 205

Tyr Leu Lys Asn Arg Leu Tyr Leu His Asn Asn Pro Leu Pro Cys Asp
    210                 215                 220

Cys Ser Leu Tyr His Leu Leu Arg Arg Trp His Gln Arg Gly Leu Ser
225                 230                 235                 240

Ala Leu His Asp Phe Glu Arg Glu Tyr Thr Cys Leu Val Phe Lys Val
                245                 250                 255

Ser Glu Ser Arg Val Arg Phe Phe Glu His Ser Arg Val Phe Lys Asn
            260                 265                 270

Cys Ser Val Ala Ala Ala Pro Gly Leu Glu Leu Pro Glu Glu Gln Leu
        275                 280                 285

His Ala Gln Val Gly Gln Ser Leu Arg Leu Phe Cys Asn Thr Ser Val
    290                 295                 300

Pro Ala Thr Arg Val Ala Trp Val Ser Pro Lys Asn Glu Leu Leu Val
305                 310                 315                 320

Ala Pro Ala Ser Gln Asp Gly Ser Ile Ala Val Leu Ala Asp Gly Ser
                325                 330                 335

Leu Ala Ile Gly Arg Val Gln Glu Gln His Ala Gly Val Phe Val Cys
            340                 345                 350

Leu Ala Ser Gly Pro Arg Leu His His Asn Gln Thr Leu Glu Tyr Asn
        355                 360                 365

Val Ser Val Gln Lys Ala Arg Pro Glu Pro Glu Thr Phe Asn Thr Gly
    370                 375                 380

Phe Thr Thr Leu Leu Gly Cys Ile Val Gly Leu Val Leu Val Leu Leu
385                 390                 395                 400

Tyr Leu Phe Ala Pro Pro Cys Arg Gly Cys Cys His Cys Cys Gln Arg
                405                 410                 415

Ala Cys Arg Asn Arg Cys Trp Pro Arg Ala Ser Ser Pro Leu Gln Glu
            420                 425                 430

Leu Ser Ala Gln Ser Ser Met Leu Ser Thr Thr Pro Pro Asp Ala Pro
        435                 440                 445

Ser Arg Lys Ala Ser Val His Lys His Val Val Phe Leu Glu Pro Gly
    450                 455                 460

Lys Lys Gly Leu Asn Gly Arg Val Gln Leu Ala Val Ala Glu Asp Phe
465                 470                 475                 480

Asp Leu Cys Asn Pro Met Gly Leu Gln Leu Lys Ala Gly Ser Glu Ser
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                485                 490                 495

Ala Ser Ser Thr Gly Ser Glu Gly Leu Val Met Ser
            500                 505
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<210> 19
<211> 3630
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(3630)

<400> 19

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atg cga ccc tcc ggg acg gcc ggg gca gcg ctc ctg gcg ctg ctg gct   48
Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu Ala
  1               5                  10                  15

gcg ctc tgc ccg gcg agt cgg gct ctg gag gaa aag aaa gtt tgc caa   96
Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys Gln
             20                  25                  30

ggc acg agt aac aag ctc acg cag ttg ggc act ttt gaa gat cat ttt   144
Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
         35                  40                  45

ctc agc ctc cag agg atg ttc aat aac tgt gag gtg gtc ctt ggg aat   192
Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
     50                  55                  60

ttg gaa att acc tat gtg cag agg aat tat gat ctt tcc ttc tta aag   240
Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
 65                  70                  75                  80

acc atc cag gag gtg gct ggt tat gtc ctc att gcc ctc aac aca gtg   288
Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
                 85                  90                  95

gag cga att cct ttg gaa aac ctg cag atc atc aga gga aat atg tac   336
Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
            100                 105                 110

tac gaa aat tcc tat gcc tta gca gtc tta tct aac tat gat gca aat   384
Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
        115                 120                 125

aaa acc gga ctg aag gag ctg ccc atg aga aat tta cag gaa atc ctg   432
Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
    130                 135                 140

cat ggc gcc gtg cgg ttc agc aac aac cct gcc ctg tgc aac gtg gag   480
His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
145                 150                 155                 160

agc atc cag tgg cgg gac ata gtc agc agt gac ttt ctc agc aac atg   528
Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
                165                 170                 175
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```
tcg atg gac ttc cag aac cac ctg ggc agc tgc caa aag tgt gat cca   576
Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
            180                 185                 190

agc tgt ccc aat ggg agc tgc tgg ggt gca gga gag gag aac tgc cag   624
Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
        195                 200                 205

aaa ctg acc aaa atc atc tgt gcc cag cag tgc tcc ggg cgc tgc cgt   672
Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg
    210                 215                 220

ggc aag tcc ccc agt gac tgc tgc cac aac cag tgt gct gca ggc tgc   720
Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
225                 230                 235                 240

aca ggc ccc cgg gag agc gac tgc ctg gtc tgc cgc aaa ttc cga gac   768
Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
                245                 250                 255

gaa gcc acg tgc aag gac acc tgc ccc cca ctc atg ctc tac aac ccc   816
Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
            260                 265                 270

acc acg tac cag atg gat gtg aac ccc gag ggc aaa tac agc ttt ggt   864
Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
        275                 280                 285

gcc acc tgc gtg aag aag tgt ccc cgt aat tat gtg gtg aca gat cac   912
Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
    290                 295                 300

ggc tcg tgc gtc cga gcc tgt ggg gcc gac agc tat gag atg gag gaa   960
Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu
305                 310                 315                 320

gac ggc gtc cgc aag tgt aag aag tgc gaa ggg cct tgc cgc aaa gtg   1008
Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
                325                 330                 335

tgt aac gga ata ggt att ggt gaa ttt aaa gac tca ctc tcc ata aat   1056
Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
            340                 345                 350

gct acg aat att aaa cac ttc aaa aac tgc acc tcc atc agt ggc gat   1104
Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
        355                 360                 365

ctc cac atc ctg ccg gtg gca ttt agg ggt gac tcc ttc aca cat act   1152
Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
    370                 375                 380

cct cct ctg gat cca cag gaa ctg gat att ctg aaa acc gta aag gaa   1200
Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
385                 390                 395                 400

atc aca ggg ttt ttg ctg att cag gct tgg cct gaa aac agg acg gac   1248
Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
                405                 410                 415

ctc cat gcc ttt gag aac cta gaa atc ata cgc ggc agg acc aag caa   1296
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Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
            420                 425                 430

cat ggt cag ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc ttg   1344
His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
        435                 440                 445

gga tta cgc tcc ctc aag gag ata agt gat gga gat gtg ata att tca   1392
Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
    450                 455                 460

gga aac aaa aat ttg tgc tat gca aat aca ata aac tgg aaa aaa ctg   1440
Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465                 470                 475                 480

ttt ggg acc tcc ggt cag aaa acc aaa att ata agc aac aga ggt gaa   1488
Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
                485                 490                 495

aac agc tgc aag gcc aca ggc cag gtc tgc cat gcc ttg tgc tcc ccc   1536
Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
            500                 505                 510

gag ggc tgc tgg ggc ccg gag ccc agg gac tgc gtc tct tgc cgg aat   1584
Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn
        515                 520                 525

gtc agc cga ggc agg gaa tgc gtg gac aag tgc aac ctt ctg gag ggt   1632
Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly
    530                 535                 540

gag cca agg gag ttt gtg gag aac tct gag tgc ata cag tgc cac cca   1680
Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545                 550                 555                 560

gag tgc ctg cct cag gcc atg aac atc acc tgc aca gga cgg gga cca   1728
Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
                565                 570                 575

gac aac tgt atc cag tgt gcc cac tac att gac ggc ccc cac tgc gtc   1776
Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
            580                 585                 590

aag acc tgc ccg gca gga gtc atg gga gaa aac aac acc ctg gtc tgg   1824
Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
        595                 600                 605

aag tac gca gac gcc ggc cat gtg tgc cac ctg tgc cat cca aac tgc   1872
Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
    610                 615                 620

acc tac gga tgc act ggg cca ggt ctt gaa ggc tgt cca acg aat ggg   1920
Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625                 630                 635                 640

cct aag atc ccg tcc atc gcc act ggg atg gtg ggg gcc ctc ctc ttg   1968
Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
                645                 650                 655

ctg ctg gtg gtg gcc ctg ggg atc ggc ctc ttc atg cga agg cgc cac   2016
Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
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            660                 665                 670

atc gtt cgg aag cgc acg ctg cgg agg ctg ctg cag gag agg gag ctt   2064
Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
        675                 680                 685

gtg gag cct ctt aca ccc agt gga gaa gct ccc aac caa gct ctc ttg   2112
Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
    690                 695                 700

agg atc ttg aag gaa act gaa ttc aaa aag atc aaa gtg ctg ggc tcc   2160
Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
705                 710                 715                 720

ggt gcg ttc ggc acg gtg tat aag gga ctc tgg atc cca gaa ggt gag   2208
Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
                725                 730                 735

aaa gtt aaa att ccc gtc gct atc aag gaa tta aga gaa gca aca tct   2256
Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
            740                 745                 750

ccg aaa gcc aac aag gaa atc ctc gat gaa gcc tac gtg atg gcc agc   2304
Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
        755                 760                 765

gtg gac aac ccc cac gtg tgc cgc ctg ctg ggc atc tgc ctc acc tcc   2352
Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
    770                 775                 780

acc gtg cag ctc atc acg cag ctc atg ccc ttc ggc tgc ctc ctg gac   2400
Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785                 790                 795                 800

tat gtc cgg gaa cac aaa gac aat att ggc tcc cag tac ctg ctc aac   2448
Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
                805                 810                 815

tgg tgt gtg cag atc gca aag ggc atg aac tac ttg gag gac cgt cgc   2496
Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
            820                 825                 830

ttg gtg cac cgc gac ctg gca gcc agg aac gta ctg gtg aaa aca ccg   2544
Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
        835                 840                 845

cag cat gtc aag atc aca gat ttt ggg ctg gcc aaa ctg ctg ggt gcg   2592
Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
    850                 855                 860

gaa gag aaa gaa tac cat gca gaa gga ggc aaa gtg cct atc aag tgg   2640
Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865                 870                 875                 880

atg gca ttg gaa tca att tta cac aga atc tat acc cac cag agt gat   2688
Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
                885                 890                 895

gtc tgg agc tac ggg gtg acc gtt tgg gag ttg atg acc ttt gga tcc   2736
Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
            900                 905                 910
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aag cca tat gac gga atc cct gcc agc gag atc tcc tcc atc ctg gag   2784
Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
        915                 920                 925

aaa gga gaa cgc ctc cct cag cca ccc ata tgt acc atc gat gtc tac   2832
Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
    930                 935                 940

atg atc atg gtc aag tgc tgg atg ata gac gca gat agt cgc cca aag   2880
Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945                 950                 955                 960

ttc cgt gag ttg atc atc gaa ttc tcc aaa atg gcc cga gac ccc cag   2928
Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975

cgc tac ctt gtc att cag ggg gat gaa aga atg cat ttg cca agt cct   2976
Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990

aca gac tcc aac ttc tac cgt gcc ctg atg gat gaa gaa gac atg gac   3024
Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
        995                1000                1005

gac gtg gtg gat gcc gac gag tac ctc atc cca cag cag ggc ttc ttc   3072
Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
   1010                1015                1020

agc agc ccc tcc acg tca cgg act ccc ctc ctg agc tct ctg agt gca   3120
Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
1025                1030                1035                1040

acc agc aac aat tcc acc gtg gct tgc att gat aga aat ggg ctg caa   3168
Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
               1045                1050                1055

agc tgt ccc atc aag gaa gac agc ttc ttg cag cga tac agc tca gac   3216
Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp
           1060                1065                1070

ccc aca ggc gcc ttg act gag gac agc ata gac gac acc ttc ctc cca   3264
Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
       1075                1080                1085

gtg cct gaa tac ata aac cag tcc gtt ccc aaa agg ccc gct ggc tct   3312
Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
   1090                1095                1100

gtg cag aat cct gtc tat cac aat cag cct ctg aac ccc gcg ccc agc   3360
Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
1105                1110                1115                1120

aga gac cca cac tac cag gac ccc cac agc act gca gtg ggc aac ccc   3408
Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
               1125                1130                1135

gag tat ctc aac act gtc cag ccc acc tgt gtc aac agc aca ttc gac   3456
Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
           1140                1145                1150
```

```
agc cct gcc cac tgg gcc cag aaa ggc agc cac caa att agc ctg gac   3504
Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp
       1155                1160                1165

aac cct gac tac cag cag gac ttc ttt ccc aag gaa gcc aag cca aat   3552
Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
   1170                1175                1180

ggc atc ttt aag ggc tcc aca gct gaa aat gca gaa tac cta agg gtc   3600
Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val
1185                1190                1195                1200

gcg cca caa agc agt gaa ttt att gga gca                          3630
Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
               1205                1210
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Met Arg Pro Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu Ala
  1               5                  10                  15

Ala Leu Cys Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys Gln
             20                  25                  30

Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
         35                  40                  45

Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
     50                  55                  60

Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
 65                  70                  75                  80

Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
                 85                  90                  95

Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
            100                 105                 110

Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
        115                 120                 125

Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
    130                 135                 140

His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
145                 150                 155                 160

Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
                165                 170                 175

Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
            180                 185                 190

Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
```

195 200 205

Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg
210 215 220

Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
225 230 235 240

Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
245 250 255

Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
260 265 270

Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
275 280 285

Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
290 295 300

Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu
305 310 315 320

Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
325 330 335

Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
340 345 350

Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
355 360 365

Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
370 375 380

Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
385 390 395 400

Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
405 410 415

Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
420 425 430

His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
435 440 445

Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
450 455 460

Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465 470 475 480

Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
485 490 495

Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
500 505 510

Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn

515 520 525

Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly
530 535 540

Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545 550 555 560

Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
565 570 575

Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
580 585 590

Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
595 600 605

Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
610 615 620

Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625 630 635 640

Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
645 650 655

Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
660 665 670

Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
675 680 685

Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
690 695 700

Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
705 710 715 720

Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
725 730 735

Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
740 745 750

Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
755 760 765

Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
770 775 780

Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785 790 795 800

Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
805 810 815

Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
820 825 830

Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
835 840 845

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Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
    850                 855                 860

Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865                 870                 875                 880

Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
                885                 890                 895

Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
            900                 905                 910

Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
        915                 920                 925

Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
    930                 935                 940

Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945                 950                 955                 960


Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975

Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990

Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
        995                1000                1005

Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
   1010                1015                1020

Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
025                1030                1035                1040

Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
               1045                1050                1055

Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp
           1060                1065                1070

Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
       1075                1080                1085

Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
   1090                1095                1100

Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
105                1110                1115                1120

Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
               1125                1130                1135

Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
           1140                1145                1150

Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp
       1155                1160                1165
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Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
   1170                1175                1180

Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val
185                1190                1195                1200

Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
               1205                1210

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gcccgagtcc ccgcctcgcc gccaacgcca caaccaccgc gcacggcccc ctgactccgt 120

ccagtattga tcgggagagc cggagcgagc tcttcgggga gcagcg atg cga ccc    175
                                                    Met Arg Pro
                                                      1

tcc ggg acg gcc ggg gca gcg ctc ctg gcg ctg ctg gct gcg ctc tgc   223
Ser Gly Thr Ala Gly Ala Ala Leu Leu Ala Leu Leu Ala Ala Leu Cys
      5                  10                  15

ccg gcg agt cgg gct ctg gag gaa aag aaa gtt tgc caa ggc acg agt   271
Pro Ala Ser Arg Ala Leu Glu Glu Lys Lys Val Cys Gln Gly Thr Ser
 20                  25                  30                  35

aac aag ctc acg cag ttg ggc act ttt gaa gat cat ttt ctc agc ctc   319
Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe Leu Ser Leu
                 40                  45                  50

cag agg atg ttc aat aac tgt gag gtg gtc ctt ggg aat ttg gaa att   367
Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn Leu Glu Ile
             55                  60                  65

acc tat gtg cag agg aat tat gat ctt tcc ttc tta aag acc atc cag   415
Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys Thr Ile Gln
         70                  75                  80

gag gtg gct ggt tat gtc ctc att gcc ctc aac aca gtg gag cga att   463
Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val Glu Arg Ile
     85                  90                  95

cct ttg gaa aac ctg cag atc atc aga gga aat atg tac tac gaa aat   511
Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr Tyr Glu Asn
100                 105                 110                 115

tcc tat gcc tta gca gtc tta tct aac tat gat gca aat aaa acc gga   559
Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn Lys Thr Gly
```

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                120                 125                 130

ctg aag gag ctg ccc atg aga aat tta cag gaa atc ctg cat ggc gcc   607
Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu His Gly Ala
            135                 140                 145

gtg cgg ttc agc aac aac cct gcc ctg tgc aac gtg gag agc atc cag   655
Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu Ser Ile Gln
        150                 155                 160

tgg cgg gac ata gtc agc agt gac ttt ctc agc aac atg tcg atg gac   703
Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met Ser Met Asp
    165                 170                 175

ttc cag aac cac ctg ggc agc tgc caa aag tgt gat cca agc tgt ccc   751
Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro Ser Cys Pro
180                 185                 190                 195

aat ggg agc tgc tgg ggt gca gga gag gag aac tgc cag aaa ctg acc   799
Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln Lys Leu Thr
                200                 205                 210

aaa atc atc tgt gcc cag cag tgc tcc ggg cgc tgc cgt ggc aag tcc   847
Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg Gly Lys Ser
            215                 220                 225

ccc agt gac tgc tgc cac aac cag tgt gct gca ggc tgc aca ggc ccc   895
Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys Thr Gly Pro
        230                 235                 240

cgg gag agc gac tgc ctg gtc tgc cgc aaa ttc cga gac gaa gcc acg   943
Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp Glu Ala Thr
    245                 250                 255

tgc aag gac acc tgc ccc cca ctc atg ctc tac aac ccc acc acg tac   991
Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro Thr Thr Tyr
260                 265                 270                 275

cag atg gat gtg aac ccc gag ggc aaa tac agc ttt ggt gcc acc tgc   1039
Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly Ala Thr Cys
                280                 285                 290

gtg aag aag tgt ccc cgt aat tat gtg gtg aca gat cac ggc tcg tgc   1087
Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His Gly Ser Cys
            295                 300                 305

gtc cga gcc tgt ggg gcc gac agc tat gag atg gag gaa gac ggc gtc   1135
Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu Asp Gly Val
        310                 315                 320

cgc aag tgt aag aag tgc gaa ggg cct tgc cgc aaa gtg tgt aac gga   1183
Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val Cys Asn Gly
    325                 330                 335

ata ggt att ggt gaa ttt aaa gac tca ctc tcc ata aat gct acg aat   1231
Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn Ala Thr Asn
340                 345                 350                 355

att aaa cac ttc aaa aac tgc acc tcc atc agt ggc gat ctc cac atc   1279
Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp Leu His Ile
                360                 365                 370
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ctg ccg gtg gca ttt agg ggt gac tcc ttc aca cat act cct cct ctg   1327
Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr Pro Pro Leu
            375                 380                 385

gat cca cag gaa ctg gat att ctg aaa acc gta aag gaa atc aca ggg   1375
Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu Ile Thr Gly
        390                 395                 400

ttt ttg ctg att cag gct tgg cct gaa aac agg acg gac ctc cat gcc   1423
Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp Leu His Ala
    405                 410                 415

ttt gag aac cta gaa atc ata cgc ggc agg acc aag caa cat ggt cag   1471
Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln His Gly Gln
420                 425                 430                 435

ttt tct ctt gca gtc gtc agc ctg aac ata aca tcc ttg gga tta cgc   1519
Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu Gly Leu Arg
                440                 445                 450

tcc ctc aag gag ata agt gat gga gat gtg ata att tca gga aac aaa   1567
Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser Gly Asn Lys
            455                 460                 465

aat ttg tgc tat gca aat aca ata aac tgg aaa aaa ctg ttt ggg acc   1615
Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu Phe Gly Thr
        470                 475                 480

tcc ggt cag aaa acc aaa att ata agc aac aga ggt gaa aac agc tgc   1663
Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu Asn Ser Cys
    485                 490                 495

aag gcc aca ggc cag gtc tgc cat gcc ttg tgc tcc ccc gag ggc tgc   1711
Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro Glu Gly Cys
500                 505                 510                 515

tgg ggc ccg gag ccc agg gac tgc gtc tct tgc cgg aat gtc agc cga   1759
Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn Val Ser Arg
                520                 525                 530

ggc agg gaa tgc gtg gac aag tgc aac ctt ctg gag ggt gag cca agg   1807
Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly Glu Pro Arg
            535                 540                 545

gag ttt gtg gag aac tct gag tgc ata cag tgc cac cca gag tgc ctg   1855
Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro Glu Cys Leu
        550                 555                 560

cct cag gcc atg aac atc acc tgc aca gga cgg gga cca gac aac tgt   1903
Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro Asp Asn Cys
    565                 570                 575

atc cag tgt gcc cac tac att gac ggc ccc cac tgc gtc aag acc tgc   1951
Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val Lys Thr Cys
580                 585                 590                 595

ccg gca gga gtc atg gga gaa aac aac acc ctg gtc tgg aag tac gca   1999
Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp Lys Tyr Ala
                600                 605                 610
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```
gac gcc ggc cat gtg tgc cac ctg tgc cat cca aac tgc acc tac gga   2047
Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys Thr Tyr Gly
            615                 620                 625

tgc act ggg cca ggt ctt gaa ggc tgt cca acg aat ggg cct aag atc   2095
Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly Pro Lys Ile
        630                 635                 640

ccg tcc atc gcc act ggg atg gtg ggg gcc ctc ctc ttg ctg ctg gtg   2143
Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu Leu Leu Val
    645                 650                 655

gtg gcc ctg ggg atc ggc ctc ttc atg cga agg cgc cac atc gtt cgg   2191
Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His Ile Val Arg
660                 665                 670                 675

aag cgc acg ctg cgg agg ctg ctg cag gag agg gag ctt gtg gag cct   2239
Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu Val Glu Pro
                680                 685                 690

ctt aca ccc agt gga gaa gct ccc aac caa gct ctc ttg agg atc ttg   2287
Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu Arg Ile Leu

            695                 700                 705

aag gaa act gaa ttc aaa aag atc aaa gtg ctg ggc tcc ggt gcg ttc   2335
Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser Gly Ala Phe
        710                 715                 720

ggc acg gtg tat aag gga ctc tgg atc cca gaa ggt gag aaa gtt aaa   2383
Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu Lys Val Lys
    725                 730                 735

att ccc gtc gct atc aag gaa tta aga gaa gca aca tct ccg aaa gcc   2431
Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser Pro Lys Ala
740                 745                 750                 755

aac aag gaa atc ctc gat gaa gcc tac gtg atg gcc agc gtg gac aac   2479
Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser Val Asp Asn
                760                 765                 770

ccc cac gtg tgc cgc ctg ctg ggc atc tgc ctc acc tcc acc gtg cag   2527
Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln
            775                 780                 785

ctc atc acg cag ctc atg ccc ttc ggc tgc ctc ctg gac tat gtc cgg   2575
Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp Tyr Val Arg
        790                 795                 800

gaa cac aaa gac aat att ggc tcc cag tac ctg ctc aac tgg tgt gtg   2623
Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn Trp Cys Val
    805                 810                 815

cag atc gca aag ggc atg aac tac ttg gag gac cgt cgc ttg gtg cac   2671
Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg Leu Val His
820                 825                 830                 835

cgc gac ctg gca gcc agg aac gta ctg gtg aaa aca ccg cag cat gtc   2719
Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro Gln His Val
                840                 845                 850
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aag atc aca gat ttt ggg ctg gcc aaa ctg ctg ggt gcg gaa gag aaa   2767
Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala Glu Glu Lys
            855                 860                 865

gaa tac cat gca gaa gga ggc aaa gtg cct atc aag tgg atg gca ttg   2815
Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu
        870                 875                 880

gaa tca att tta cac aga atc tat acc cac cag agt gat gtc tgg agc   2863
Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp Val Trp Ser
    885                 890                 895

tac ggg gtg acc gtt tgg gag ttg atg acc ttt gga tcc aag cca tat   2911
Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser Lys Pro Tyr
900                 905                 910                 915

gac gga atc cct gcc agc gag atc tcc tcc atc ctg gag aaa gga gaa   2959
Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu Lys Gly Glu
                920                 925                 930

cgc ctc cct cag cca ccc ata tgt acc atc gat gtc tac atg atc atg   3007
Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met
            935                 940                 945

gtc aag tgc tgg atg ata gac gca gat agt cgc cca aag ttc cgt gag   3055
Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe Arg Glu
        950                 955                 960

ttg atc atc gaa ttc tcc aaa atg gcc cga gac ccc cag cgc tac ctt   3103
Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln Arg Tyr Leu
    965                 970                 975

gtc att cag ggg gat gaa aga atg cat ttg cca agt cct aca gac tcc   3151
Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro Thr Asp Ser
980                 985                 990                 995

aac ttc tac cgt gcc ctg atg gat gaa gaa gac atg gac gac gtg gtg   3199
Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp Asp Val Val
                1000                1005                1010

gat gcc gac gag tac ctc atc cca cag cag ggc ttc ttc agc agc ccc   3247
Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe Ser Ser Pro
            1015                1020                1025

tcc acg tca cgg act ccc ctc ctg agc tct ctg agt gca acc agc aac   3295
Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala Thr Ser Asn
        1030                1035                1040

aat tcc acc gtg gct tgc att gat aga aat ggg ctg caa agc tgt ccc   3343
Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln Ser Cys Pro
    1045                1050                1055

atc aag gaa gac agc ttc ttg cag cga tac agc tca gac ccc aca ggc   3391
Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp Pro Thr Gly
1060                1065                1070                1075

gcc ttg act gag gac agc ata gac gac acc ttc ctc cca gtg cct gaa   3439
Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro Val Pro Glu
                1080                1085                1090

tac ata aac cag tcc gtt ccc aaa agg ccc gct ggc tct gtg cag aat   3487
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Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser Val Gln Asn
          1095                1100                1105

cct gtc tat cac aat cag cct ctg aac ccc gcg ccc agc aga gac cca   3535
Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser Arg Asp Pro
       1110                1115                1120

cac tac cag gac ccc cac agc act gca gtg ggc aac ccc gag tat ctc   3583
His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro Glu Tyr Leu
   1125                1130                1135

aac act gtc cag ccc acc tgt gtc aac agc aca ttc gac agc cct gcc   3631
Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp Ser Pro Ala
1140                1145                1150                1155

cac tgg gcc cag aaa ggc agc cac caa att agc ctg gac aac cct gac   3679
His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp Asn Pro Asp
               1160                1165                1170

tac cag cag gac ttc ttt ccc aag gaa gcc aag cca aat ggc atc ttt   3727
Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn Gly Ile Phe
          1175                1180                1185

aag ggc tcc aca gct gaa aat gca gaa tac cta agg gtc gcg cca caa   3775
Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val Ala Pro Gln
       1190                1195                1200

agc agt gaa ttt att gga gca tga ccacggagga tagtatgagc cctaaaaatc  3829
Ser Ser Glu Phe Ile Gly Ala
   1205                1210

cagactcttt cgatacccag gaccaagcca cagcaggtcc tccatcccaa cagccatgcc 3889

cgcattagct cttagaccca cagactggtt ttgcaacgtt tacaccgact agccaggaag 3949

tacttccacc tcgggcacat tttgggaagt tgcattcctt tgtcttcaaa ctgtgaagca 4009

tttacagaaa cgcatccagc aagaatattg tccctttgag cagaaattta tctttcaaag 4069

aggtatattt gaaaaaaaaa aaaaaagtat atgtgaggat ttttattgat tggggatctt 4129

ggagtttttc attgtcgcta ttgatttta cttcaatggg ctcttccaac aaggaagaag 4189

cttgctggta gcacttgcta ccctgagttc atccaggccc aactgtgagc aaggagcaca 4249

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gaggggatga attcttcctt agacttactt ttgtaaaaat gtccccacgg tacttactcc 4489

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ccattgtttt gaaactcagt atgccgcccc tgtcttgctg tcatgaaatc agcaagagag 4609

gatgacacat caaataataa ctcggattcc agcccacatt ggattcatca gcatttggac 4669

caatagccca cagctgagaa tgtggaatac ctaaggataa caccgctttt gttctcgcaa 4729
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aaacgtatct cctaatttga ggctcagatg aaatgcatca ggtcctttgg ggcatagatc 4789

agaagactac aaaaatgaag ctgctctgaa atctccttta gccatcaccc caaccccccа 4849

aaattagttt gtgttactta tggaagatag ttttctcctt ttacttcact tcaaaagctt 4909

tttactcaaa gagtatatgt tccctccagg tcagctgccc ccaaaccccc tccttacgct 4969

ttgtcacaca aaaagtgtct ctgccttgag tcatctattc aagcacttac agctctggcc 5029

acaacagggc attttacagg tgcgaatgac agtagcatta tgagtagtgt gaattcaggt 5089

agtaaatatg aaactagggt ttgaaattga taatgctttc acaacatttg cagatgtttt 5149

agaaggaaaa aagttccttc ctaaaataat ttctctacaa ttggaagatt ggaagattca 5209

gctagttagg agcccatttt ttcctaatct gtgtgtgccc tgtaacctga ctggttaaca 5269

gcagtccttt gtaaacagtg ttttaaactc tcctagtcaa tatccacccc atccaattta 5329

tcaaggaaga aatggttcag aaaatatttt cagcctacag ttatgttcag tcacacacac 5389

atacaaaatg ttccttttgc ttttaaagta atttttgact cccagatcag tcagagcccc 5449

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tcc 5512

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             20                  25                  30
Gly Thr Ser Asn Lys Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
         35                  40                  45
Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
     50                  55                  60
Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
 65                  70                  75                  80
Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
                 85                  90                  95
Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
            100                 105                 110
Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
        115                 120                 125
Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
    130                 135                 140
His Gly Ala Val Arg Phe Ser Asn Asn Pro Ala Leu Cys Asn Val Glu
145                 150                 155                 160
Ser Ile Gln Trp Arg Asp Ile Val Ser Ser Asp Phe Leu Ser Asn Met
                165                 170                 175
Ser Met Asp Phe Gln Asn His Leu Gly Ser Cys Gln Lys Cys Asp Pro
            180                 185                 190
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Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
        195                 200                 205
Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg
    210                 215                 220
Gly Lys Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
225                 230                 235                 240
Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Arg Lys Phe Arg Asp
                245                 250                 255
Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
            260                 265                 270
Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
        275                 280                 285
Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
    290                 295                 300
Gly Ser Cys Val Arg Ala Cys Gly Ala Asp Ser Tyr Glu Met Glu Glu
305                 310                 315                 320
Asp Gly Val Arg Lys Cys Lys Lys Cys Glu Gly Pro Cys Arg Lys Val
                325                 330                 335
Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
            340                 345                 350
Ala Thr Asn Ile Lys His Phe Lys Asn Cys Thr Ser Ile Ser Gly Asp
        355                 360                 365
Leu His Ile Leu Pro Val Ala Phe Arg Gly Asp Ser Phe Thr His Thr
    370                 375                 380
Pro Pro Leu Asp Pro Gln Glu Leu Asp Ile Leu Lys Thr Val Lys Glu
385                 390                 395                 400
Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Glu Asn Arg Thr Asp
                405                 410                 415
Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
            420                 425                 430
His Gly Gln Phe Ser Leu Ala Val Val Ser Leu Asn Ile Thr Ser Leu
        435                 440                 445
Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
    450                 455                 460
Gly Asn Lys Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465                 470                 475                 480
Phe Gly Thr Ser Gly Gln Lys Thr Lys Ile Ile Ser Asn Arg Gly Glu
                485                 490                 495
Asn Ser Cys Lys Ala Thr Gly Gln Val Cys His Ala Leu Cys Ser Pro
            500                 505                 510
Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Arg Asn
        515                 520                 525
Val Ser Arg Gly Arg Glu Cys Val Asp Lys Cys Asn Leu Leu Glu Gly
    530                 535                 540
Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545                 550                 555                 560
Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
                565                 570                 575
Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
            580                 585                 590
Lys Thr Cys Pro Ala Gly Val Met Gly Glu Asn Asn Thr Leu Val Trp
        595                 600                 605
Lys Tyr Ala Asp Ala Gly His Val Cys His Leu Cys His Pro Asn Cys
    610                 615                 620
Thr Tyr Gly Cys Thr Gly Pro Gly Leu Glu Gly Cys Pro Thr Asn Gly
625                 630                 635                 640
Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
                645                 650                 655
Leu Leu Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His
            660                 665                 670
Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu
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        675                 680                 685
Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
    690                 695                 700
Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
705                 710                 715                 720
Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
                725                 730                 735
Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
            740                 745                 750
Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser
        755                 760                 765
Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser
    770                 775                 780
Thr Val Gln Leu Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp
785                 790                 795                 800
Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
                805                 810                 815
Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
            820                 825                 830
Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
        835                 840                 845
Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
    850                 855                 860
Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865                 870                 875                 880
Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
                885                 890                 895
Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
            900                 905                 910
Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
        915                 920                 925
Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
    930                 935                 940
Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945                 950                 955                 960
Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975
Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990
Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Asp
        995                1000                1005
Asp Val Val Asp Ala Asp Glu Tyr Leu Ile Pro Gln Gln Gly Phe Phe
   1010                1015                1020
Ser Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala
1025                1030                1035                1040
Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asp Arg Asn Gly Leu Gln
                1045                1050                1055
Ser Cys Pro Ile Lys Glu Asp Ser Phe Leu Gln Arg Tyr Ser Ser Asp
            1060                1065                1070
Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp Asp Thr Phe Leu Pro
        1075                1080                1085
Val Pro Glu Tyr Ile Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
   1090                1095                1100
Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu Asn Pro Ala Pro Ser
1105                1110                1115                1120
Arg Asp Pro His Tyr Gln Asp Pro His Ser Thr Ala Val Gly Asn Pro
                1125                1130                1135
Glu Tyr Leu Asn Thr Val Gln Pro Thr Cys Val Asn Ser Thr Phe Asp
            1140                1145                1150
Ser Pro Ala His Trp Ala Gln Lys Gly Ser His Gln Ile Ser Leu Asp
        1155                1160                1165
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Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Ala Lys Pro Asn
   1170                1175                1180
Gly Ile Phe Lys Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val
1185               1190                1195                1200
Ala Pro Gln Ser Ser Glu Phe Ile Gly Ala
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tccgggtctc gtcgttgccg gcgcagtcac tgggcacaac cgtgggactc cgtctgtctc 180

ggattaatcc cggagagcca gagccaacct ctcccggtca gag atg cga ccc tca 235
                                                Met Arg Pro Ser
                                                  1

ggg acc gcg aga acc aca ctg ctg gtg ctg ctg acc gcg ctc tgc gcc 283
Gly Thr Ala Arg Thr Thr Leu Leu Val Leu Leu Thr Ala Leu Cys Ala
  5   10   15   20

gca ggt ggg gcg ttg gag gaa aag aaa gtc tgc caa ggc aca agt aac 331
Ala Gly Gly Ala Leu Glu Glu Lys Lys Val Cys Gln Gly Thr Ser Asn
                 25   30   35

agg ctc acc caa ctg ggc act ttt gaa gac cac ttt ctg agc ctg cag 379
Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe Leu Ser Leu Gln
             40   45   50

agg atg tac aac aac tgt gaa gtg gtc ctt ggg aac ttg gaa att acc 427
Arg Met Tyr Asn Asn Cys Glu Val Val Leu Gly Asn Leu Glu Ile Thr
         55   60   65

tat gtg caa agg aat tac gac ctt tcc ttc tta aag acc atc cag gag 475
Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys Thr Ile Gln Glu
     70   75   80

gtg gcc ggc tat gtc ctc att gcc ctc aac acc gtg gag aga atc cct 523
Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val Glu Arg Ile Pro
 85   90   95   100

ttg gag aac ctg cag atc atc agg gga aat gct ctt tat gaa aac acc 571
Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Ala Leu Tyr Glu Asn Thr
                105   110   115

tat gcc tta gcc atc ctg tcc aac tat ggg aca aac aga act ggg ctt 619

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Tyr Ala Leu Ala Ile Leu Ser Asn Tyr Gly Thr Asn Arg Thr Gly Leu
            120                 125                 130

agg gaa ctg ccc atg cgg aac tta cag gaa atc ctg att ggt gct gtg   667
Arg Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu Ile Gly Ala Val
        135                 140                 145

cga ttc agc aac aac ccc atc ctc tgc aat atg gat act atc cag tgg   715
Arg Phe Ser Asn Asn Pro Ile Leu Cys Asn Met Asp Thr Ile Gln Trp
    150                 155                 160

agg gac atc gtc caa aac gtc ttt atg agc aac atg tca atg gac tta   763
Arg Asp Ile Val Gln Asn Val Phe Met Ser Asn Met Ser Met Asp Leu
165                 170                 175                 180

cag agc cat ccg agc agt tgc ccc aaa tgt gat cca agc tgt ccc aat   811
Gln Ser His Pro Ser Ser Cys Pro Lys Cys Asp Pro Ser Cys Pro Asn
                185                 190                 195

gga agc tgc tgg gga gga gga gag gag aac tgc cag aaa ttg acc aaa   859
Gly Ser Cys Trp Gly Gly Gly Glu Glu Asn Cys Gln Lys Leu Thr Lys
            200                 205                 210

atc atc tgt gcc cag caa tgt tcc cat cgc tgt cgt ggc agg tcc ccc   907
Ile Ile Cys Ala Gln Gln Cys Ser His Arg Cys Arg Gly Arg Ser Pro
        215                 220                 225

agt gac tgc tgc cac aac caa tgt gct gcg ggg tgt aca ggg ccc cga   955
Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys Thr Gly Pro Arg
    230                 235                 240

gag agt gac tgt ctg gtc tgc caa aag ttc caa gat gag gcc aca tgc   1003
Glu Ser Asp Cys Leu Val Cys Gln Lys Phe Gln Asp Glu Ala Thr Cys
245                 250                 255                 260

aaa gac acc tgc cca cca ctc atg ctg tac aac ccc acc acc tat cag   1051
Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro Thr Thr Tyr Gln
                265                 270                 275

atg gat gtc aac cct gaa ggg aag tac agc ttt ggt gcc acc tgt gtg   1099
Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly Ala Thr Cys Val
            280                 285                 290

aag aag tgc ccc cga aac tac gtg gtg aca gat cat ggc tca tgt gtc   1147
Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His Gly Ser Cys Val
        295                 300                 305

cga gcc tgt ggg cct gac tac tac gaa gtg gaa gaa gat ggc atc cgc   1195
Arg Ala Cys Gly Pro Asp Tyr Tyr Glu Val Glu Glu Asp Gly Ile Arg
    310                 315                 320

aag tgt aaa aaa tgt gat ggg ccc tgt cgc aaa gtt tgt aat ggc ata   1243
Lys Cys Lys Lys Cys Asp Gly Pro Cys Arg Lys Val Cys Asn Gly Ile
325                 330                 335                 340

ggc att ggt gaa ttt aaa gac aca ctc tcc ata aat gct aca aac atc   1291
Gly Ile Gly Glu Phe Lys Asp Thr Leu Ser Ile Asn Ala Thr Asn Ile
                345                 350                 355

aaa cac ttc aaa tac tgc act gcc atc agc ggg gac ctt cac atc ctg   1339
Lys His Phe Lys Tyr Cys Thr Ala Ile Ser Gly Asp Leu His Ile Leu
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            360                 365                 370

cca gtg gcc ttt aag ggg gat tct ttc acg cgc act cct cct cta gac   1387
Pro Val Ala Phe Lys Gly Asp Ser Phe Thr Arg Thr Pro Pro Leu Asp
        375                 380                 385

cca cga gaa cta gaa att cta aaa acc gta aag gaa ata aca ggc ttt   1435
Pro Arg Glu Leu Glu Ile Leu Lys Thr Val Lys Glu Ile Thr Gly Phe
    390                 395                 400

ttg ctg att cag gct tgg cct gat aac tgg act gac ctc cat gct ttc   1483
Leu Leu Ile Gln Ala Trp Pro Asp Asn Trp Thr Asp Leu His Ala Phe
405                 410                 415                 420

gag aac cta gaa ata ata cgt ggc aga aca aag caa cat ggt cag ttt   1531
Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln His Gly Gln Phe
                425                 430                 435

tct ttg gcg gtc gtt ggc ctg aac atc aca tca ctg ggg ctg cgt tcc   1579
Ser Leu Ala Val Val Gly Leu Asn Ile Thr Ser Leu Gly Leu Arg Ser
            440                 445                 450

ctc aag gag atc agt gat ggg gat gtg atc att tct gga aac cga aat   1627
Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser Gly Asn Arg Asn
        455                 460                 465

ttg tgc tac gca aac aca ata aac tgg aaa aaa ctc ttc ggg aca ccc   1675
Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu Phe Gly Thr Pro
    470                 475                 480

aat cag aaa acc aaa atc atg aac aac aga gct gag aaa gac tgc aag   1723
Asn Gln Lys Thr Lys Ile Met Asn Asn Arg Ala Glu Lys Asp Cys Lys
485                 490                 495                 500

gcc gtg aac cac gtc tgc aat cct tta tgc tcc tcg gaa ggc tgc tgg   1771
Ala Val Asn His Val Cys Asn Pro Leu Cys Ser Ser Glu Gly Cys Trp
                505                 510                 515

ggc cct gag ccc agg gac tgt gtc tcc tgc cag aat gtg agc aga ggc   1819
Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Gln Asn Val Ser Arg Gly
            520                 525                 530

agg gag tgc gtg gag aaa tgc aac atc ctg gag ggg gaa cca agg gag   1867
Arg Glu Cys Val Glu Lys Cys Asn Ile Leu Glu Gly Glu Pro Arg Glu
        535                 540                 545

ttt gtg gaa aat tct gaa tgc atc cag tgc cat cca gaa tgt ctg ccc   1915
Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro Glu Cys Leu Pro
    550                 555                 560

cag gcc atg aac atc acc tgt aca ggc agg ggg cca gac aac tgc atc   1963
Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro Asp Asn Cys Ile
565                 570                 575                 580

cag tgt gcc cac tac att gat ggc cca cac tgt gtc aag acc tgc cca   2011
Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val Lys Thr Cys Pro
                585                 590                 595

gct ggc atc atg gga gag aac aac act ctg gtc tgg aag tat gca gat   2059
Ala Gly Ile Met Gly Glu Asn Asn Thr Leu Val Trp Lys Tyr Ala Asp
            600                 605                 610
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gcc aat aat gtc tgc cac cta tgc cac gcc aac tgt acc tat gga tgt   2107
Ala Asn Asn Val Cys His Leu Cys His Ala Asn Cys Thr Tyr Gly Cys
        615                 620                 625

gct ggg cca ggt ctt caa gga tgt gaa gtg tgg cca tct ggg cca aag   2155
Ala Gly Pro Gly Leu Gln Gly Cys Glu Val Trp Pro Ser Gly Pro Lys
    630                 635                 640

ata cca tct att gcc act ggg att gtg ggt ggc ctc ctc ttc ata gtg   2203
Ile Pro Ser Ile Ala Thr Gly Ile Val Gly Gly Leu Leu Phe Ile Val
645                 650                 655                 660

gtg gtg gcc ctt ggg att ggc cta ttc atg cga aga cgt cac att gtt   2251
Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg Arg His Ile Val
                665                 670                 675

cga aag cgt aca cta cgc cgc ctg ctt caa gag aga gag ctc gtg gaa   2299
Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg Glu Leu Val Glu
            680                 685                 690

cct ctc aca ccc agc gga gaa gct cca aac caa gcc cac ttg agg ata   2347
Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala His Leu Arg Ile
        695                 700                 705

tta aag gaa aca gaa ttc aaa aag atc aaa gtt ctg ggt tcg gga gca   2395
Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser Gly Ala
    710                 715                 720

ttt ggc aca gtg tat aag ggt ctc tgg atc cca gaa ggt gag aaa gta   2443
Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu Lys Val
725                 730                 735                 740

aaa atc ccg gtg gcc atc aag gag tta aga gaa gcc aca tct cca aaa   2491
Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser Pro Lys
                745                 750                 755

gcc aac aaa gaa atc ctt gac gaa gcc tat gtg atg gct agt gtg gac   2539
Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met Ala Ser Val Asp
            760                 765                 770

aac cct cat gta tgc cgc ctc ctg ggc atc tgt ctg acc tcc act gtc   2587
Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu Thr Ser Thr Val
        775                 780                 785

cag ctc att aca cag ctc atg ccc tac ggt tgc ctc ctg gac tac gtc   2635
Gln Leu Ile Thr Gln Leu Met Pro Tyr Gly Cys Leu Leu Asp Tyr Val
    790                 795                 800

cga gaa cac aag gac aac att ggc tcc cag tac ctc ctc aac tgg tgt   2683
Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn Trp Cys
805                 810                 815                 820

gtg cag att gca aag ggc atg aac tac ctg gaa gat cgg cgt ttg gtg   2731
Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg Leu Val
                825                 830                 835

cac cgt gac ttg gca gcc agg aat gta ctg gtg aag aca cca cag cat   2779
His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro Gln His
            840                 845                 850
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gtc aag atc aca gat ttt ggg ctg gcc aaa ctg ctt ggt gct gaa gag   2827
Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala Glu Glu
        855                 860                 865

aaa gaa tat cat gcc gag ggg ggc aaa gtg cct atc aag tgg atg gct   2875
Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp Met Ala

    870                 875                 880

ttg gaa tca att tta cac cga att tat aca cac caa agt gat gtc tgg   2923
Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp Val Trp
885                 890                 895                 900

agc tat ggt gtc act gtg tgg gaa ctg atg acc ttt ggg tcc aag cct   2971
Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser Lys Pro
                905                 910                 915

tat gat gga atc cca gca agt gac atc tca tcc atc cta gag aaa gga   3019
Tyr Asp Gly Ile Pro Ala Ser Asp Ile Ser Ser Ile Leu Glu Lys Gly
            920                 925                 930

gaa cgc ctt cca cag cca cct atc tgc acc atc gat gtc tac atg atc   3067
Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met Ile
        935                 940                 945

atg gtc aag tgc tgg atg ata gat gct gat agc cgc cca aag ttc cga   3115
Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe Arg
    950                 955                 960

gag ttg att ctt gaa ttc tcc aaa atg gcc cga gac cca cag cgc tac   3163
Glu Leu Ile Leu Glu Phe Ser Lys Met Ala Arg Asp Pro Gln Arg Tyr
965                 970                 975                 980

ctt gtt atc cag ggg gat gaa aga atg cat ttg cca agc cct aca gac   3211
Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro Thr Asp
                985                 990                 995

tcc aac ttt tac cga gcc ctg atg gat gaa gag gac atg gag gat gta   3259
Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp Met Glu Asp Val
            1000                1005                1010

gtt gat gct gat gag tat ctt acc cca cag caa ggc ttc ttc aac agc   3307
Val Asp Ala Asp Glu Tyr Leu Thr Pro Gln Gln Gly Phe Phe Asn Ser
        1015                1020                1025

ccg tcc acg tcg agg act ccc ctc ttg agt tct ctg agt gca act agc   3355
Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu Ser Ala Thr Ser
    1030                1035                1040

aac aat tcc act gtg gct tgc att aat aga aat ggg agc tgc cgt gtc   3403
Asn Asn Ser Thr Val Ala Cys Ile Asn Arg Asn Gly Ser Cys Arg Val
1045                1050                1055                1060

aaa gaa gac gcc ttc ttg cag cgg tac agc tcc gac ccc aca ggt gct   3451
Lys Glu Asp Ala Phe Leu Gln Arg Tyr Ser Ser Asp Pro Thr Gly Ala
                1065                1070                1075

gta aca gag gac aac ata gat gac gca ttc ctc cct gta cct gaa tat   3499
Val Thr Glu Asp Asn Ile Asp Asp Ala Phe Leu Pro Val Pro Glu Tyr
            1080                1085                1090
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gta aac caa tct gtt ccc aag agg cca gca ggc tct gtg cag aac cct   3547
Val Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser Val Gln Asn Pro
       1095                1100                1105

gtc tat cac aat cag ccc ctg cat cca gct cct gga aga gac ctg cat   3595
Val Tyr His Asn Gln Pro Leu His Pro Ala Pro Gly Arg Asp Leu His
   1110                1115                1120

tat caa aat ccc cac agc aat gca gtg ggc aac cct gag tat ctc aac   3643
Tyr Gln Asn Pro His Ser Asn Ala Val Gly Asn Pro Glu Tyr Leu Asn
1125                1130                1135                1140

act gcc cag cct acc tgt ctc agt agt ggg ttt aac agc cct gca ctc   3691
Thr Ala Gln Pro Thr Cys Leu Ser Ser Gly Phe Asn Ser Pro Ala Leu
               1145                1150                1155

tgg atc cag aaa ggc agt cac caa atg agc cta gac aac cct gac tac   3739
Trp Ile Gln Lys Gly Ser His Gln Met Ser Leu Asp Asn Pro Asp Tyr
           1160                1165                1170

cag cag gac ttc ttc ccc aag gaa acc aag cca aat ggc ata ttt aag   3787
Gln Gln Asp Phe Phe Pro Lys Glu Thr Lys Pro Asn Gly Ile Phe Lys
       1175                1180                1185

ggc ccc aca gct gaa aat gca gag tac cta cgg gtg gca cct cca agc   3835
Gly Pro Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val Ala Pro Pro Ser
   1190                1195                1200

agc gag ttt att gga gca tga caagaagggg catcatacca gctataaaat      3886
Ser Glu Phe Ile Gly Ala
1205                1210

gtctggactt tctagaatcc caggaccaac tatggcagca cctccacttc tggtagccat 3946

gcccacgctg tgtcaaatgt cactcagact ggctttaaag cataactctg atgggctttg 4006

tcactgagcc aagaagtggg cctctctcct gatgcacttt gggaagttga aggtacatca 4066

attgatcttc gaactgtgaa gattccacaa aaaaggtatc catcgagaac attgtccatt 4126

ggaacagaag tttgcctcat ggtgaggtac atatgggaaa aaaacagaca tatggagctt 4186

atgtttaggg aactttggga ttcttgtctt tattgatttg attgatgcac tcttgtagtc 4246

tggtacacag agttgcctgg agccaactga ccagacagtt ggttccacca gctctgcatc 4306

aagacacttc cgtggcaaga caactaaatg tataagaagt ccatggatgc cctgagcagg 4366

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ctatgttttc tgtttcattg ttttgagttg taaatatgtg ttcctgtctt catttcatga 4606

agtaaacaaa caaacaaaaa acccagtatt aagtattatc aaagaacaac catgattcca 4666

cattcgaacc cattcaaacc atcagtattg tgaccaaaag cctttaacta agaaggagta 4726

accatgcaaa aatccataga ggaatttaac ccaaaatttt agtctcagca ttgtgtctgc 4786
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tgatggatag acgtgaactc ataaacacta cccataagac aaaacaatga gtgccagaca 5086

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agaaccgttg caagccactc taactgtagc aatgaaaccc tagtattttt gtactttgaa 5866

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Gly Thr Ser Asn Arg Leu Thr Gln Leu Gly Thr Phe Glu Asp His Phe
        35                  40                  45
Leu Ser Leu Gln Arg Met Tyr Asn Asn Cys Glu Val Val Leu Gly Asn
    50                  55                  60
Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
65                  70                  75                  80
Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
                85                  90                  95
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Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Ala Leu
            100                 105                 110
Tyr Glu Asn Thr Tyr Ala Leu Ala Ile Leu Ser Asn Tyr Gly Thr Asn
        115                 120                 125
Arg Thr Gly Leu Arg Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
    130                 135                 140
Ile Gly Ala Val Arg Phe Ser Asn Asn Pro Ile Leu Cys Asn Met Asp
145                 150                 155                 160
Thr Ile Gln Trp Arg Asp Ile Val Gln Asn Val Phe Met Ser Asn Met
                165                 170                 175
Ser Met Asp Leu Gln Ser His Pro Ser Ser Cys Pro Lys Cys Asp Pro
            180                 185                 190
Ser Cys Pro Asn Gly Ser Cys Trp Gly Gly Gly Glu Glu Asn Cys Gln
        195                 200                 205
Lys Leu Thr Lys Ile Ile Cys Ala Gln Gln Cys Ser His Arg Cys Arg
    210                 215                 220
Gly Arg Ser Pro Ser Asp Cys Cys His Asn Gln Cys Ala Ala Gly Cys
225                 230                 235                 240
Thr Gly Pro Arg Glu Ser Asp Cys Leu Val Cys Gln Lys Phe Gln Asp
                245                 250                 255
Glu Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Met Leu Tyr Asn Pro
            260                 265                 270
Thr Thr Tyr Gln Met Asp Val Asn Pro Glu Gly Lys Tyr Ser Phe Gly
        275                 280                 285
Ala Thr Cys Val Lys Lys Cys Pro Arg Asn Tyr Val Val Thr Asp His
    290                 295                 300
Gly Ser Cys Val Arg Ala Cys Gly Pro Asp Tyr Tyr Glu Val Glu Glu
305                 310                 315                 320
Asp Gly Ile Arg Lys Cys Lys Lys Cys Asp Gly Pro Cys Arg Lys Val
                325                 330                 335
Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Thr Leu Ser Ile Asn
            340                 345                 350
Ala Thr Asn Ile Lys His Phe Lys Tyr Cys Thr Ala Ile Ser Gly Asp
        355                 360                 365
Leu His Ile Leu Pro Val Ala Phe Lys Gly Asp Ser Phe Thr Arg Thr
    370                 375                 380
Pro Pro Leu Asp Pro Arg Glu Leu Glu Ile Leu Lys Thr Val Lys Glu
385                 390                 395                 400
Ile Thr Gly Phe Leu Leu Ile Gln Ala Trp Pro Asp Asn Trp Thr Asp
                405                 410                 415
Leu His Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln
            420                 425                 430
His Gly Gln Phe Ser Leu Ala Val Val Gly Leu Asn Ile Thr Ser Leu
        435                 440                 445
Gly Leu Arg Ser Leu Lys Glu Ile Ser Asp Gly Asp Val Ile Ile Ser
    450                 455                 460
Gly Asn Arg Asn Leu Cys Tyr Ala Asn Thr Ile Asn Trp Lys Lys Leu
465                 470                 475                 480
Phe Gly Thr Pro Asn Gln Lys Thr Lys Ile Met Asn Asn Arg Ala Glu
                485                 490                 495
Lys Asp Cys Lys Ala Val Asn His Val Cys Asn Pro Leu Cys Ser Ser
            500                 505                 510
Glu Gly Cys Trp Gly Pro Glu Pro Arg Asp Cys Val Ser Cys Gln Asn
        515                 520                 525
Val Ser Arg Gly Arg Glu Cys Val Glu Lys Cys Asn Ile Leu Glu Gly
    530                 535                 540
Glu Pro Arg Glu Phe Val Glu Asn Ser Glu Cys Ile Gln Cys His Pro
545                 550                 555                 560
Glu Cys Leu Pro Gln Ala Met Asn Ile Thr Cys Thr Gly Arg Gly Pro
                565                 570                 575
Asp Asn Cys Ile Gln Cys Ala His Tyr Ile Asp Gly Pro His Cys Val
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            580                 585                 590
Lys Thr Cys Pro Ala Gly Ile Met Gly Glu Asn Asn Thr Leu Val Trp
        595                 600                 605
Lys Tyr Ala Asp Ala Asn Asn Val Cys His Leu Cys His Ala Asn Cys
    610                 615                 620
Thr Tyr Gly Cys Ala Gly Pro Gly Leu Gln Gly Cys Glu Val Trp Pro
625                 630                 635                 640
Ser Gly Pro Lys Ile Pro Ser Ile Ala Thr Gly Ile Val Gly Gly Leu
                645                 650                 655
Leu Phe Ile Val Val Val Ala Leu Gly Ile Gly Leu Phe Met Arg Arg
            660                 665                 670
Arg His Ile Val Arg Lys Arg Thr Leu Arg Arg Leu Leu Gln Glu Arg
        675                 680                 685
Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala
    690                 695                 700
His Leu Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu
705                 710                 715                 720
Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu
                725                 730                 735
Gly Glu Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala
            740                 745                 750
Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu Asp Glu Ala Tyr Val Met
        755                 760                 765
Ala Ser Val Asp Asn Pro His Val Cys Arg Leu Leu Gly Ile Cys Leu
    770                 775                 780
Thr Ser Thr Val Gln Leu Ile Thr Gln Leu Met Pro Tyr Gly Cys Leu
785                 790                 795                 800
Leu Asp Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu
                805                 810                 815
Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp
            820                 825                 830
Arg Arg Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys
        835                 840                 845
Thr Pro Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu
    850                 855                 860
Gly Ala Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile
865                 870                 875                 880
Lys Trp Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln
                885                 890                 895
Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe
            900                 905                 910
Gly Ser Lys Pro Tyr Asp Gly Ile Pro Ala Ser Asp Ile Ser Ser Ile
        915                 920                 925
Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp
    930                 935                 940
Val Tyr Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg
945                 950                 955                 960
Pro Lys Phe Arg Glu Leu Ile Leu Glu Phe Ser Lys Met Ala Arg Asp
                965                 970                 975
Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro
            980                 985                 990
Ser Pro Thr Asp Ser Asn Phe Tyr Arg Ala Leu Met Asp Glu Glu Asp
        995                1000                1005
Met Glu Asp Val Val Asp Ala Asp Glu Tyr Leu Thr Pro Gln Gln Gly
   1010                1015                1020
Phe Phe Asn Ser Pro Ser Thr Ser Arg Thr Pro Leu Leu Ser Ser Leu
1025                1030                1035                1040
Ser Ala Thr Ser Asn Asn Ser Thr Val Ala Cys Ile Asn Arg Asn Gly
                1045                1050                1055
Ser Cys Arg Val Lys Glu Asp Ala Phe Leu Gln Arg Tyr Ser Ser Asp
            1060                1065                1070
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Pro Thr Gly Ala Val Thr Glu Asp Asn Ile Asp Asp Ala Phe Leu Pro
       1075                1080                1085
Val Pro Glu Tyr Val Asn Gln Ser Val Pro Lys Arg Pro Ala Gly Ser
   1090                1095                1100

Val Gln Asn Pro Val Tyr His Asn Gln Pro Leu His Pro Ala Pro Gly
1105                1110                1115                1120
Arg Asp Leu His Tyr Gln Asn Pro His Ser Asn Ala Val Gly Asn Pro
                1125                1130                1135
Glu Tyr Leu Asn Thr Ala Gln Pro Thr Cys Leu Ser Ser Gly Phe Asn
            1140                1145                1150
Ser Pro Ala Leu Trp Ile Gln Lys Gly Ser His Gln Met Ser Leu Asp
       1155                1160                1165
Asn Pro Asp Tyr Gln Gln Asp Phe Phe Pro Lys Glu Thr Lys Pro Asn
   1170                1175                1180
Gly Ile Phe Lys Gly Pro Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val
1185                1190                1195                1200
Ala Pro Pro Ser Ser Glu Phe Ile Gly Ala
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gtcacagtga agtcagccag agcagggctg ttaaactctg tgaaatttgt cataagggtg 180

tcaggtattt cttactggct tccaaagaaa catagataaa gaaatctttc ctgtggcttc 240

ccttggcagg ctgcattcag aaggtctctc agttgaagaa agagcttgga ggacaacagc 300

acaacaggag agtaaaagat gccccagggc tgaggcctcc gctcaggcag ccgcatctgg 360

ggtcaatcat actcaccttg cccgggccat gctccagcaa aatcaagctg ttttcttttg 420

aaagttcaaa ctcatcaaga tt atg ctg ctc act ctt atc att ctg ttg cca 472
                          Met Leu Leu Thr Leu Ile Ile Leu Leu Pro
                            1   5   10

gta gtt tca aaa ttt agt ttt gtt agt ctc tca gca ccg cag cac tgg 520
Val Val Ser Lys Phe Ser Phe Val Ser Leu Ser Ala Pro Gln His Trp
                 15   20   25

agc tgt cct gaa ggt act ctc gca gga aat ggg aat tct act tgt gtg 568
Ser Cys Pro Glu Gly Thr Leu Ala Gly Asn Gly Asn Ser Thr Cys Val
             30   35   40

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ggt cct gca ccc ttc tta att ttc tcc cat gga aat agt atc ttt agg   616
Gly Pro Ala Pro Phe Leu Ile Phe Ser His Gly Asn Ser Ile Phe Arg
         45                  50                  55

att gac aca gaa gga acc aat tat gag caa ttg gtg gtg gat gct ggt   664
Ile Asp Thr Glu Gly Thr Asn Tyr Glu Gln Leu Val Val Asp Ala Gly
     60                  65                  70

gtc tca gtg atc atg gat ttt cat tat aat gag aaa aga atc tat tgg   712
Val Ser Val Ile Met Asp Phe His Tyr Asn Glu Lys Arg Ile Tyr Trp
 75                  80                  85                  90

gtg gat tta gaa aga caa ctt ttg caa aga gtt ttt ctg aat ggg tca   760
Val Asp Leu Glu Arg Gln Leu Leu Gln Arg Val Phe Leu Asn Gly Ser
                95                 100                 105

agg caa gag aga gta tgt aat ata gag aaa aat gtt tct gga atg gca   808
Arg Gln Glu Arg Val Cys Asn Ile Glu Lys Asn Val Ser Gly Met Ala
            110                 115                 120

ata aat tgg ata aat gaa gaa gtt att tgg tca aat caa cag gaa gga   856
Ile Asn Trp Ile Asn Glu Glu Val Ile Trp Ser Asn Gln Gln Glu Gly
        125                 130                 135

atc att aca gta aca gat atg aaa gga aat aat tcc cac att ctt tta   904
Ile Ile Thr Val Thr Asp Met Lys Gly Asn Asn Ser His Ile Leu Leu
    140                 145                 150

agt gct tta aaa tat cct gca aat gta gca gtt gat cca gta gaa agg   952
Ser Ala Leu Lys Tyr Pro Ala Asn Val Ala Val Asp Pro Val Glu Arg
155                 160                 165                 170

ttt ata ttt tgg tct tca gag gtg gct gga agc ctt tat aga gca gat  1000
Phe Ile Phe Trp Ser Ser Glu Val Ala Gly Ser Leu Tyr Arg Ala Asp
                175                 180                 185

ctc gat ggt gtg gga gtg aag gct ctg ttg gag aca tca gag aaa ata  1048
Leu Asp Gly Val Gly Val Lys Ala Leu Leu Glu Thr Ser Glu Lys Ile
            190                 195                 200

aca gct gtg tca ttg gat gtg ctt gat aag cgg ctg ttt tgg att cag  1096
Thr Ala Val Ser Leu Asp Val Leu Asp Lys Arg Leu Phe Trp Ile Gln
        205                 210                 215

tac aac aga gaa gga agc aat tct ctt att tgc tcc tgt gat tat gat  1144
Tyr Asn Arg Glu Gly Ser Asn Ser Leu Ile Cys Ser Cys Asp Tyr Asp
    220                 225                 230

gga ggt tct gtc cac att agt aaa cat cca aca cag cat aat ttg ttt  1192
Gly Gly Ser Val His Ile Ser Lys His Pro Thr Gln His Asn Leu Phe
235                 240                 245                 250

gca atg tcc ctt ttt ggt gac cgt atc ttc tat tca aca tgg aaa atg  1240
Ala Met Ser Leu Phe Gly Asp Arg Ile Phe Tyr Ser Thr Trp Lys Met
                255                 260                 265

aag aca att tgg ata gcc aac aaa cac act gga aag gac atg gtt aga  1288
Lys Thr Ile Trp Ile Ala Asn Lys His Thr Gly Lys Asp Met Val Arg
            270                 275                 280

att aac ctc cat tca tca ttt gta cca ctt ggt gaa ctg aaa gta gtg  1336
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Ile Asn Leu His Ser Ser Phe Val Pro Leu Gly Glu Leu Lys Val Val
        285                 290                 295

cat cca ctt gca caa ccc aag gca gaa gat gac act tgg gag cct gag   1384
His Pro Leu Ala Gln Pro Lys Ala Glu Asp Asp Thr Trp Glu Pro Glu
    300                 305                 310

cag aaa ctt tgc aaa ttg agg aaa gga aac tgc agc agc act gtg tgt   1432
Gln Lys Leu Cys Lys Leu Arg Lys Gly Asn Cys Ser Ser Thr Val Cys
315                 320                 325                 330

ggg caa gac ctc cag tca cac ttg tgc atg tgt gca gag gga tac gcc   1480
Gly Gln Asp Leu Gln Ser His Leu Cys Met Cys Ala Glu Gly Tyr Ala
                335                 340                 345

cta agt cga gac cgg aag tac tgt gaa gat gtt aat gaa tgt gct ttt   1528
Leu Ser Arg Asp Arg Lys Tyr Cys Glu Asp Val Asn Glu Cys Ala Phe
            350                 355                 360

tgg aat cat ggc tgt act ctt ggg tgt aaa aac acc cct gga tcc tat   1576
Trp Asn His Gly Cys Thr Leu Gly Cys Lys Asn Thr Pro Gly Ser Tyr
        365                 370                 375

tac tgc acg tgc cct gta gga ttt gtt ctg ctt cct gat ggg aaa cga   1624
Tyr Cys Thr Cys Pro Val Gly Phe Val Leu Leu Pro Asp Gly Lys Arg
    380                 385                 390

tgt cat caa ctt gtt tcc tgt cca cgc aat gtg tct gaa tgc agc cat   1672
Cys His Gln Leu Val Ser Cys Pro Arg Asn Val Ser Glu Cys Ser His
395                 400                 405                 410

gac tgt gtt ctg aca tca gaa ggt ccc tta tgt ttc tgt cct gaa ggc   1720
Asp Cys Val Leu Thr Ser Glu Gly Pro Leu Cys Phe Cys Pro Glu Gly
                415                 420                 425

tca gtg ctt gag aga gat ggg aaa aca tgt agc ggt tgt tcc tca ccc   1768
Ser Val Leu Glu Arg Asp Gly Lys Thr Cys Ser Gly Cys Ser Ser Pro
            430                 435                 440

gat aat ggt gga tgt agc cag ctc tgc gtt cct ctt agc cca gta tcc   1816
Asp Asn Gly Gly Cys Ser Gln Leu Cys Val Pro Leu Ser Pro Val Ser
        445                 450                 455

tgg gaa tgt gat tgc ttt cct ggg tat gac cta caa ctg gat gaa aaa   1864
Trp Glu Cys Asp Cys Phe Pro Gly Tyr Asp Leu Gln Leu Asp Glu Lys
    460                 465                 470

agc tgt gca gct tca gga cca caa cca ttt ttg ctg ttt gcc aat tct   1912
Ser Cys Ala Ala Ser Gly Pro Gln Pro Phe Leu Leu Phe Ala Asn Ser
475                 480                 485                 490

caa gat att cga cac atg cat ttt gat gga aca gac tat gga act ctg   1960
Gln Asp Ile Arg His Met His Phe Asp Gly Thr Asp Tyr Gly Thr Leu
                495                 500                 505

ctc agc cag cag atg gga atg gtt tat gcc cta gat cat gac cct gtg   2008
Leu Ser Gln Gln Met Gly Met Val Tyr Ala Leu Asp His Asp Pro Val
            510                 515                 520

gaa aat aag ata tac ttt gcc cat aca gcc ctg aag tgg ata gag aga   2056
Glu Asn Lys Ile Tyr Phe Ala His Thr Ala Leu Lys Trp Ile Glu Arg
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        525                 530                 535

gct aat atg gat ggt tcc cag cga gaa agg ctt att gag gaa gga gta   2104
Ala Asn Met Asp Gly Ser Gln Arg Glu Arg Leu Ile Glu Glu Gly Val
    540                 545                 550

gat gtg cca gaa ggt ctt gct gtg gac tgg att ggc cgt aga ttc tat   2152
Asp Val Pro Glu Gly Leu Ala Val Asp Trp Ile Gly Arg Arg Phe Tyr
555                 560                 565                 570

tgg aca gac aga ggg aaa tct ctg att gga agg agt gat tta aat ggg   2200
Trp Thr Asp Arg Gly Lys Ser Leu Ile Gly Arg Ser Asp Leu Asn Gly
                575                 580                 585

aaa cgt tcc aaa ata atc act aag gag aac atc tct caa cca cga gga   2248
Lys Arg Ser Lys Ile Ile Thr Lys Glu Asn Ile Ser Gln Pro Arg Gly
            590                 595                 600

att gct gtt cat cca atg gcc aag aga tta ttc tgg act gat aca ggg   2296
Ile Ala Val His Pro Met Ala Lys Arg Leu Phe Trp Thr Asp Thr Gly
        605                 610                 615

att aat cca cga att gaa agt tct tcc ctc caa ggc ctt ggc cgt ctg   2344
Ile Asn Pro Arg Ile Glu Ser Ser Ser Leu Gln Gly Leu Gly Arg Leu
    620                 625                 630

gtt ata gcc agc tct gat cta atc tgg ccc agt gga ata acg att gac   2392
Val Ile Ala Ser Ser Asp Leu Ile Trp Pro Ser Gly Ile Thr Ile Asp
635                 640                 645                 650

ttc tta act gac aag ttg tac tgg tgc gat gcc aag cag tct gtg att   2440
Phe Leu Thr Asp Lys Leu Tyr Trp Cys Asp Ala Lys Gln Ser Val Ile
                655                 660                 665

gaa atg gcc aat ctg gat ggt tca aaa cgc cga aga ctt acc cag aat   2488
Glu Met Ala Asn Leu Asp Gly Ser Lys Arg Arg Arg Leu Thr Gln Asn
            670                 675                 680

gat gta ggt cac cca ttt gct gta gca gtg ttt gag gat tat gtg tgg   2536
Asp Val Gly His Pro Phe Ala Val Ala Val Phe Glu Asp Tyr Val Trp
        685                 690                 695

ttc tca gat tgg gct atg cca tca gta ata aga gta aac aag agg act   2584
Phe Ser Asp Trp Ala Met Pro Ser Val Ile Arg Val Asn Lys Arg Thr
    700                 705                 710

ggc aaa gat aga gta cgt ctc caa ggc agc atg ctg aag ccc tca tca   2632
Gly Lys Asp Arg Val Arg Leu Gln Gly Ser Met Leu Lys Pro Ser Ser
715                 720                 725                 730

ctg gtt gtg gtt cat cca ttg gca aaa cca gga gca gat ccc tgc tta   2680
Leu Val Val Val His Pro Leu Ala Lys Pro Gly Ala Asp Pro Cys Leu
                735                 740                 745

tat caa aac gga ggc tgt gaa cat att tgc aaa aag agg ctt gga act   2728
Tyr Gln Asn Gly Gly Cys Glu His Ile Cys Lys Lys Arg Leu Gly Thr
            750                 755                 760

gct tgg tgt tcg tgt cgt gaa ggt ttt atg aaa gcc tca gat ggg aaa   2776
Ala Trp Cys Ser Cys Arg Glu Gly Phe Met Lys Ala Ser Asp Gly Lys
        765                 770                 775
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acg tgt ctg gct ctg gat ggt cat cag ctg ttg gca ggt ggt gaa gtt   2824
Thr Cys Leu Ala Leu Asp Gly His Gln Leu Leu Ala Gly Gly Glu Val
    780                 785                 790

gat cta aag aac caa gta aca cca ttg gac atc ttg tcc aag act aga   2872
Asp Leu Lys Asn Gln Val Thr Pro Leu Asp Ile Leu Ser Lys Thr Arg
795                 800                 805                 810

gtg tca gaa gat aac att aca gaa tct caa cac atg cta gtg gct gaa   2920
Val Ser Glu Asp Asn Ile Thr Glu Ser Gln His Met Leu Val Ala Glu
                815                 820                 825

atc atg gtg tca gat caa gat gac tgt gct cct gtg gga tgc agc atg   2968
Ile Met Val Ser Asp Gln Asp Asp Cys Ala Pro Val Gly Cys Ser Met
            830                 835                 840

tat gct cgg tgt att tca gag gga gag gat gcc aca tgt cag tgt ttg   3016
Tyr Ala Arg Cys Ile Ser Glu Gly Glu Asp Ala Thr Cys Gln Cys Leu
        845                 850                 855

aaa gga ttt gct ggg gat gga aaa cta tgt tct gat ata gat gaa tgt   3064
Lys Gly Phe Ala Gly Asp Gly Lys Leu Cys Ser Asp Ile Asp Glu Cys
    860                 865                 870

gag atg ggt gtc cca gtg tgc ccc cct gcc tcc tcc aag tgc atc aac   3112
Glu Met Gly Val Pro Val Cys Pro Pro Ala Ser Ser Lys Cys Ile Asn
875                 880                 885                 890

acc gaa ggt ggt tat gtc tgc cgg tgc tca gaa ggc tac caa gga gat   3160
Thr Glu Gly Gly Tyr Val Cys Arg Cys Ser Glu Gly Tyr Gln Gly Asp
                895                 900                 905

ggg att cac tgt ctt gat att gat gag tgc caa ctg ggg gtg cac agc   3208
Gly Ile His Cys Leu Asp Ile Asp Glu Cys Gln Leu Gly Val His Ser
            910                 915                 920

tgt gga gag aat gcc agc tgc aca aat aca gag gga ggc tat acc tgc   3256
Cys Gly Glu Asn Ala Ser Cys Thr Asn Thr Glu Gly Gly Tyr Thr Cys
        925                 930                 935

atg tgt gct gga cgc ctg tct gaa cca gga ctg att tgc cct gac tct   3304
Met Cys Ala Gly Arg Leu Ser Glu Pro Gly Leu Ile Cys Pro Asp Ser
    940                 945                 950

act cca ccc cct cac ctc agg gaa gat gac cac cac tat tcc gta aga   3352
Thr Pro Pro Pro His Leu Arg Glu Asp Asp His His Tyr Ser Val Arg
955                 960                 965                 970

aat agt gac tct gaa tgt ccc ctg tcc cac gat ggg tac tgc ctc cat   3400
Asn Ser Asp Ser Glu Cys Pro Leu Ser His Asp Gly Tyr Cys Leu His
                975                 980                 985

gat ggt gtg tgc atg tat att gaa gca ttg gac aag tat gca tgc aac   3448
Asp Gly Val Cys Met Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn
            990                 995                1000

tgt gtt gtt ggc tac atc ggg gag cga tgt cag tac cga gac ctg aag   3496
Cys Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys
       1005                1010                1015
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tgg tgg gaa ctg cgc cac gct ggc cac ggg cag cag cag aag gtc atc   3544
Trp Trp Glu Leu Arg His Ala Gly His Gly Gln Gln Gln Lys Val Ile
   1020                1025                1030

gtg gtg gct gtc tgc gtg gtg gtg ctt gtc atg ctg ctc ctc ctg agc   3592
Val Val Ala Val Cys Val Val Val Leu Val Met Leu Leu Leu Leu Ser
1035               1040                1045                1050

ctg tgg ggg gcc cac tac tac agg act cag aag ctg cta tcg aaa aac   3640
Leu Trp Gly Ala His Tyr Tyr Arg Thr Gln Lys Leu Leu Ser Lys Asn
              1055                1060                1065

cca aag aat cct tat gag gag tcg agc aga gat gtg agg agt cgc agg   3688
Pro Lys Asn Pro Tyr Glu Glu Ser Ser Arg Asp Val Arg Ser Arg Arg
          1070                1075                1080


cct gct gac act gag gat ggg atg tcc tct tgc cct caa cct tgg ttt   3736
Pro Ala Asp Thr Glu Asp Gly Met Ser Ser Cys Pro Gln Pro Trp Phe
       1085                1090                1095

gtg gtt ata aaa gaa cac caa gac ctc aag aat ggg ggt caa cca gtg   3784
Val Val Ile Lys Glu His Gln Asp Leu Lys Asn Gly Gly Gln Pro Val
   1100                1105                1110

gct ggt gag gat ggc cag gca gca gat ggg tca atg caa cca act tca   3832
Ala Gly Glu Asp Gly Gln Ala Ala Asp Gly Ser Met Gln Pro Thr Ser
1115               1120                1125                1130

tgg agg cag gag ccc cag tta tgt gga atg ggc aca gag caa ggc tgc   3880
Trp Arg Gln Glu Pro Gln Leu Cys Gly Met Gly Thr Glu Gln Gly Cys
              1135                1140                1145

tgg att cca gta tcc agt gat aag ggc tcc tgt ccc cag gta atg gag   3928
Trp Ile Pro Val Ser Ser Asp Lys Gly Ser Cys Pro Gln Val Met Glu
          1150                1155                1160

cga agc ttt cat atg ccc tcc tat ggg aca cag acc ctt gaa ggg ggt   3976
Arg Ser Phe His Met Pro Ser Tyr Gly Thr Gln Thr Leu Glu Gly Gly
       1165                1170                1175

gtc gag aag ccc cat tct ctc cta tca gct aac cca tta tgg caa caa   4024
Val Glu Lys Pro His Ser Leu Leu Ser Ala Asn Pro Leu Trp Gln Gln
   1180                1185                1190

agg gcc ctg gac cca cca cac caa atg gag ctg act cag tga           4066
Arg Ala Leu Asp Pro Pro His Gln Met Glu Leu Thr Gln
1195               1200                1205

aaactggaat taaaaggaaa gtcaagaaga atgaactatg tcgatgcaca gtatcttttc 4126

tttcaaaagt agagcaaaac tataggtttt ggttccacaa tctctacgac taatcaccta 4186

ctcaatgcct ggagacagat acgtagttgt gcttttgttt gctcttttaa gcagtctcac 4246

tgcagtctta tttccaagta agagtactgg gagaatcact aggtaactta ttagaaaccc 4306

aaattgggac aacagtgctt tgtaaattgt gttgtcttca gcagtcaata caaatagatt 4366

tttgtttttg ttgttcctgc agccccagaa gaaattaggg gttaaagcag acagtcacac 4426
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tggtttggtc agttacaaag taatttcttt gatctggaca gaacatttat atcagtttca 4486

tgaaatgatt ggaatattac aataccgtta agatacagtg taggcattta actcctcatt 4546

ggcgtggtcc atgctgatga ttttgccaaa atgagttgtg atgaatcaat gaaaaatgta 4606

atttagaaac tgatttcttc agaattagat ggccttattt tttaaaatat ttgaatgaaa 4666

acattttatt tttaaaatat tacacaggag gccttcggag tttcttagtc attactgtcc 4726

ttttccccta cagaattttc cctcttggtg tgattgcaca gaatttgtat gtattttcag 4786

ttacaagatt gtaagtaaat tgcctgattt gttttcatta tagacaacga tgaatttctt 4846

ctaattattt aaataaaatc accaaaaaca t 4877

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<223> Human EGF

<400> 26

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Met Leu Leu Thr Leu Ile Ile Leu Leu Pro Val Val Ser Lys Phe Ser
  1               5                  10                  15
Phe Val Ser Leu Ser Ala Pro Gln His Trp Ser Cys Pro Glu Gly Thr
             20                  25                  30
Leu Ala Gly Asn Gly Asn Ser Thr Cys Val Gly Pro Ala Pro Phe Leu
         35                  40                  45
Ile Phe Ser His Gly Asn Ser Ile Phe Arg Ile Asp Thr Glu Gly Thr
     50                  55                  60
Asn Tyr Glu Gln Leu Val Val Asp Ala Gly Val Ser Val Ile Met Asp
 65                  70                  75                  80
Phe His Tyr Asn Glu Lys Arg Ile Tyr Trp Val Asp Leu Glu Arg Gln
                 85                  90                  95
Leu Leu Gln Arg Val Phe Leu Asn Gly Ser Arg Gln Glu Arg Val Cys
            100                 105                 110
Asn Ile Glu Lys Asn Val Ser Gly Met Ala Ile Asn Trp Ile Asn Glu
        115                 120                 125
Glu Val Ile Trp Ser Asn Gln Gln Glu Gly Ile Ile Thr Val Thr Asp
    130                 135                 140
Met Lys Gly Asn Asn Ser His Ile Leu Leu Ser Ala Leu Lys Tyr Pro
145                 150                 155                 160
Ala Asn Val Ala Val Asp Pro Val Glu Arg Phe Ile Phe Trp Ser Ser
                165                 170                 175
Glu Val Ala Gly Ser Leu Tyr Arg Ala Asp Leu Asp Gly Val Gly Val
            180                 185                 190
Lys Ala Leu Leu Glu Thr Ser Glu Lys Ile Thr Ala Val Ser Leu Asp
        195                 200                 205
Val Leu Asp Lys Arg Leu Phe Trp Ile Gln Tyr Asn Arg Glu Gly Ser
    210                 215                 220
Asn Ser Leu Ile Cys Ser Cys Asp Tyr Asp Gly Gly Ser Val His Ile
225                 230                 235                 240
Ser Lys His Pro Thr Gln His Asn Leu Phe Ala Met Ser Leu Phe Gly

                245                 250                 255
Asp Arg Ile Phe Tyr Ser Thr Trp Lys Met Lys Thr Ile Trp Ile Ala
            260                 265                 270
Asn Lys His Thr Gly Lys Asp Met Val Arg Ile Asn Leu His Ser Ser
        275                 280                 285
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Phe Val Pro Leu Gly Glu Leu Lys Val Val His Pro Leu Ala Gln Pro
    290                 295                 300
Lys Ala Glu Asp Asp Thr Trp Glu Pro Glu Gln Lys Leu Cys Lys Leu
305                 310                 315                 320
Arg Lys Gly Asn Cys Ser Ser Thr Val Cys Gly Gln Asp Leu Gln Ser
                325                 330                 335
His Leu Cys Met Cys Ala Glu Gly Tyr Ala Leu Ser Arg Asp Arg Lys
            340                 345                 350
Tyr Cys Glu Asp Val Asn Glu Cys Ala Phe Trp Asn His Gly Cys Thr
        355                 360                 365
Leu Gly Cys Lys Asn Thr Pro Gly Ser Tyr Tyr Cys Thr Cys Pro Val
    370                 375                 380
Gly Phe Val Leu Leu Pro Asp Gly Lys Arg Cys His Gln Leu Val Ser
385                 390                 395                 400
Cys Pro Arg Asn Val Ser Glu Cys Ser His Asp Cys Val Leu Thr Ser
                405                 410                 415
Glu Gly Pro Leu Cys Phe Cys Pro Glu Gly Ser Val Leu Glu Arg Asp
            420                 425                 430
Gly Lys Thr Cys Ser Gly Cys Ser Ser Pro Asp Asn Gly Gly Cys Ser
        435                 440                 445
Gln Leu Cys Val Pro Leu Ser Pro Val Ser Trp Glu Cys Asp Cys Phe
    450                 455                 460
Pro Gly Tyr Asp Leu Gln Leu Asp Glu Lys Ser Cys Ala Ala Ser Gly
465                 470                 475                 480
Pro Gln Pro Phe Leu Leu Phe Ala Asn Ser Gln Asp Ile Arg His Met
                485                 490                 495
His Phe Asp Gly Thr Asp Tyr Gly Thr Leu Leu Ser Gln Gln Met Gly
            500                 505                 510
Met Val Tyr Ala Leu Asp His Asp Pro Val Glu Asn Lys Ile Tyr Phe
        515                 520                 525
Ala His Thr Ala Leu Lys Trp Ile Glu Arg Ala Asn Met Asp Gly Ser
    530                 535                 540
Gln Arg Glu Arg Leu Ile Glu Glu Gly Val Asp Val Pro Glu Gly Leu
545                 550                 555                 560
Ala Val Asp Trp Ile Gly Arg Arg Phe Tyr Trp Thr Asp Arg Gly Lys
                565                 570                 575
Ser Leu Ile Gly Arg Ser Asp Leu Asn Gly Lys Arg Ser Lys Ile Ile
            580                 585                 590
Thr Lys Glu Asn Ile Ser Gln Pro Arg Gly Ile Ala Val His Pro Met
        595                 600                 605
Ala Lys Arg Leu Phe Trp Thr Asp Thr Gly Ile Asn Pro Arg Ile Glu
    610                 615                 620
Ser Ser Ser Leu Gln Gly Leu Gly Arg Leu Val Ile Ala Ser Ser Asp
625                 630                 635                 640
Leu Ile Trp Pro Ser Gly Ile Thr Ile Asp Phe Leu Thr Asp Lys Leu
                645                 650                 655
Tyr Trp Cys Asp Ala Lys Gln Ser Val Ile Glu Met Ala Asn Leu Asp
            660                 665                 670
Gly Ser Lys Arg Arg Arg Leu Thr Gln Asn Asp Val Gly His Pro Phe
        675                 680                 685
Ala Val Ala Val Phe Glu Asp Tyr Val Trp Phe Ser Asp Trp Ala Met
    690                 695                 700
Pro Ser Val Ile Arg Val Asn Lys Arg Thr Gly Lys Asp Arg Val Arg
705                 710                 715                 720
Leu Gln Gly Ser Met Leu Lys Pro Ser Ser Leu Val Val Val His Pro
                725                 730                 735
Leu Ala Lys Pro Gly Ala Asp Pro Cys Leu Tyr Gln Asn Gly Gly Cys
            740                 745                 750
Glu His Ile Cys Lys Lys Arg Leu Gly Thr Ala Trp Cys Ser Cys Arg
        755                 760                 765
Glu Gly Phe Met Lys Ala Ser Asp Gly Lys Thr Cys Leu Ala Leu Asp
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    770                 775                 780
Gly His Gln Leu Leu Ala Gly Gly Glu Val Asp Leu Lys Asn Gln Val
785                 790                 795                 800
Thr Pro Leu Asp Ile Leu Ser Lys Thr Arg Val Ser Glu Asp Asn Ile
                805                 810                 815
Thr Glu Ser Gln His Met Leu Val Ala Glu Ile Met Val Ser Asp Gln
            820                 825                 830
Asp Asp Cys Ala Pro Val Gly Cys Ser Met Tyr Ala Arg Cys Ile Ser
        835                 840                 845
Glu Gly Glu Asp Ala Thr Cys Gln Cys Leu Lys Gly Phe Ala Gly Asp
    850                 855                 860
Gly Lys Leu Cys Ser Asp Ile Asp Glu Cys Glu Met Gly Val Pro Val
865                 870                 875                 880
Cys Pro Pro Ala Ser Ser Lys Cys Ile Asn Thr Glu Gly Gly Tyr Val
                885                 890                 895
Cys Arg Cys Ser Glu Gly Tyr Gln Gly Asp Gly Ile His Cys Leu Asp
            900                 905                 910
Ile Asp Glu Cys Gln Leu Gly Val His Ser Cys Gly Glu Asn Ala Ser
        915                 920                 925
Cys Thr Asn Thr Glu Gly Gly Tyr Thr Cys Met Cys Ala Gly Arg Leu
    930                 935                 940
Ser Glu Pro Gly Leu Ile Cys Pro Asp Ser Thr Pro Pro Pro His Leu
945                 950                 955                 960
Arg Glu Asp Asp His His Tyr Ser Val Arg Asn Ser Asp Ser Glu Cys
                965                 970                 975
Pro Leu Ser His Asp Gly Tyr Cys Leu His Asp Gly Val Cys Met Tyr
            980                 985                 990
Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn Cys Val Val Gly Tyr Ile
        995                1000                1005
Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp Trp Glu Leu Arg His
   1010                1015                1020
Ala Gly His Gly Gln Gln Gln Lys Val Ile Val Val Ala Val Cys Val
1025                1030                1035                1040
Val Val Leu Val Met Leu Leu Leu Leu Ser Leu Trp Gly Ala His Tyr
                1045                1050                1055
Tyr Arg Thr Gln Lys Leu Leu Ser Lys Asn Pro Lys Asn Pro Tyr Glu
            1060                1065                1070
Glu Ser Ser Arg Asp Val Arg Ser Arg Arg Pro Ala Asp Thr Glu Asp
        1075                1080                1085
Gly Met Ser Ser Cys Pro Gln Pro Trp Phe Val Val Ile Lys Glu His
   1090                1095                1100
Gln Asp Leu Lys Asn Gly Gly Gln Pro Val Ala Gly Glu Asp Gly Gln
1105                1110                1115                1120
Ala Ala Asp Gly Ser Met Gln Pro Thr Ser Trp Arg Gln Glu Pro Gln
                1125                1130                1135
Leu Cys Gly Met Gly Thr Glu Gln Gly Cys Trp Ile Pro Val Ser Ser
            1140                1145                1150
Asp Lys Gly Ser Cys Pro Gln Val Met Glu Arg Ser Phe His Met Pro
        1155                1160                1165
Ser Tyr Gly Thr Gln Thr Leu Glu Gly Gly Val Glu Lys Pro His Ser
   1170                1175                1180
Leu Leu Ser Ala Asn Pro Leu Trp Gln Gln Arg Ala Leu Asp Pro Pro
1185                1190                1195                1200
His Gln Met Glu Leu Thr Gln
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tgttagcacc atccctcatc ccggtgggct tggaactttc catcaattct ttcctgtctc 120

gtttctcttt catcctttgc ctggttgtgc ctgtctcagg gagaaatcag tcacctgcag 180

gccttgcagg gctcttaggc tctgggaaat ttgtcatacg ggtgtcaggt acttcttatt 240

gctgtccaaa gggaaaaaaa aagtgagaca aagaactctc ccggagcctt tccggctgca 300

ctcagaggct ctcgagaggt gcagaaggac ctggaaaggc agctaaataa aag atg 356
Met
1

ccc tgg ggc cga agg cca acc tgg ctg ttg ctc gcc ttc ctg ctg gtg 404
Pro Trp Gly Arg Arg Pro Thr Trp Leu Leu Leu Ala Phe Leu Leu Val
5 10 15

ttt tta aag att agc ata ctc agc gtc aca gca tgg cag acc ggg aac 452
Phe Leu Lys Ile Ser Ile Leu Ser Val Thr Ala Trp Gln Thr Gly Asn
20 25 30

tgt cag cca ggt cct ctc gag aga agc gag aga agc ggg act tgt gcc 500
Cys Gln Pro Gly Pro Leu Glu Arg Ser Glu Arg Ser Gly Thr Cys Ala
35 40 45

ggt cct gcc ccc ttc cta gtt ttc tca caa gga aag agc atc tct cgg 548
Gly Pro Ala Pro Phe Leu Val Phe Ser Gln Gly Lys Ser Ile Ser Arg
50 55 60 65

att gac cca gat gga aca aat cac cag caa ttg gtg gtg gat gct ggc 596

Ile Asp Pro Asp Gly Thr Asn His Gln Gln Leu Val Val Asp Ala Gly
70 75 80

atc tca gca gac atg gat att cat tat aaa aaa gag aga ctc tat tgg 644
Ile Ser Ala Asp Met Asp Ile His Tyr Lys Lys Glu Arg Leu Tyr Trp
85 90 95

gtg gat gta gaa aga caa gtt ttg cta aga gtt ttc ctt aac ggg aca 692
Val Asp Val Glu Arg Gln Val Leu Leu Arg Val Phe Leu Asn Gly Thr
100 105 110

gga cta gag aaa gtg tgc aat gta gag agg aag gtg tct ggg ctg gcc 740
Gly Leu Glu Lys Val Cys Asn Val Glu Arg Lys Val Ser Gly Leu Ala
115 120 125

ata gac tgg ata gat gat gaa gtt ctc tgg gta gac caa cag aac gga 788
Ile Asp Trp Ile Asp Asp Glu Val Leu Trp Val Asp Gln Gln Asn Gly
130 135 140 145

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gtc atc acc gta aca gat atg aca ggg aaa aat tcc cga gtt ctt cta   836
Val Ile Thr Val Thr Asp Met Thr Gly Lys Asn Ser Arg Val Leu Leu
                150                 155                 160

agt tcc tta aaa cat ccg tca aat ata gca gtg gat cca ata gag agg   884
Ser Ser Leu Lys His Pro Ser Asn Ile Ala Val Asp Pro Ile Glu Arg
            165                 170                 175

ttg atg ttt tgg tct tca gag gtg acc ggc agc ctt cac aga gca cac   932
Leu Met Phe Trp Ser Ser Glu Val Thr Gly Ser Leu His Arg Ala His
        180                 185                 190

ctc aaa ggt gtt gat gta aaa aca ctg ctg gag aca ggg gga ata tcg   980
Leu Lys Gly Val Asp Val Lys Thr Leu Leu Glu Thr Gly Gly Ile Ser
    195                 200                 205

gtg ctg act ctg gat gtc ctg gac aaa cgg ctc ttc tgg gtt cag gac   1028
Val Leu Thr Leu Asp Val Leu Asp Lys Arg Leu Phe Trp Val Gln Asp
210                 215                 220                 225

agt ggc gaa gga agc cac gct tac att cat tcc tgt gat tat gag ggt   1076
Ser Gly Glu Gly Ser His Ala Tyr Ile His Ser Cys Asp Tyr Glu Gly
                230                 235                 240

ggc tcc gtc cgt ctt atc agg cat caa gca cgg cac agt ttg tct tca   1124
Gly Ser Val Arg Leu Ile Arg His Gln Ala Arg His Ser Leu Ser Ser
            245                 250                 255

atg gcc ttt ttt ggt gat cgg atc ttc tac tca gtg ttg aaa agc aag   1172
Met Ala Phe Phe Gly Asp Arg Ile Phe Tyr Ser Val Leu Lys Ser Lys
        260                 265                 270

gcg att tgg ata gcc aac aaa cac acg ggg aag gac acg gtc agg att   1220
Ala Ile Trp Ile Ala Asn Lys His Thr Gly Lys Asp Thr Val Arg Ile
    275                 280                 285

aac ctc cat cca tcc ttt gtg aca cct gga aaa ctg atg gta gta cac   1268
Asn Leu His Pro Ser Phe Val Thr Pro Gly Lys Leu Met Val Val His
290                 295                 300                 305

cct cgt gca cag ccc agg aca gag gac gct gct aag gat cct gac ccc   1316
Pro Arg Ala Gln Pro Arg Thr Glu Asp Ala Ala Lys Asp Pro Asp Pro
                310                 315                 320

gaa ctt ctc aaa cag agg gga aga cca tgc cgc ttc ggt ctc tgt gag   1364
Glu Leu Leu Lys Gln Arg Gly Arg Pro Cys Arg Phe Gly Leu Cys Glu
            325                 330                 335

cga gac ccc aag tcc cac tcg agc gca tgc gct gag ggc tac acg tta   1412
Arg Asp Pro Lys Ser His Ser Ser Ala Cys Ala Glu Gly Tyr Thr Leu
        340                 345                 350

agc cga gac cgg aag tac tgc gaa gat gtc aat gaa tgt gcc act cag   1460
Ser Arg Asp Arg Lys Tyr Cys Glu Asp Val Asn Glu Cys Ala Thr Gln
    355                 360                 365


aat cac ggc tgt act ctt ggg tgt gaa aac acc cct gga tcc tat cac   1508
Asn His Gly Cys Thr Leu Gly Cys Glu Asn Thr Pro Gly Ser Tyr His
370                 375                 380                 385
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tgc aca tgc ccc aca gga ttt gtt ctg ctt cct gat ggg aaa caa tgt   1556
Cys Thr Cys Pro Thr Gly Phe Val Leu Leu Pro Asp Gly Lys Gln Cys
                390                 395                 400

cac gaa ctt gtt tcc tgc cca ggc aac gta tca aag tgc agt cat ggc   1604
His Glu Leu Val Ser Cys Pro Gly Asn Val Ser Lys Cys Ser His Gly
            405                 410                 415

tgt gtc ctg aca tca gat ggt ccc cgg tgc atc tgt cct gca ggt tca   1652
Cys Val Leu Thr Ser Asp Gly Pro Arg Cys Ile Cys Pro Ala Gly Ser
        420                 425                 430

gtg ctt ggg aga gat ggg aag act tgc act ggt tgt tca tcg cct gac   1700
Val Leu Gly Arg Asp Gly Lys Thr Cys Thr Gly Cys Ser Ser Pro Asp
    435                 440                 445

aat ggt gga tgc agc cag atc tgt ctt cct ctc agg cca gga tcc tgg   1748
Asn Gly Gly Cys Ser Gln Ile Cys Leu Pro Leu Arg Pro Gly Ser Trp
450                 455                 460                 465

gaa tgt gat tgc ttt cct ggg tat gac cta cag tca gac cga aag agc   1796
Glu Cys Asp Cys Phe Pro Gly Tyr Asp Leu Gln Ser Asp Arg Lys Ser
                470                 475                 480

tgt gca gct tca gga cca cag cca ctt tta ctg ttt gca aat tcc cag   1844
Cys Ala Ala Ser Gly Pro Gln Pro Leu Leu Leu Phe Ala Asn Ser Gln
            485                 490                 495

gac atc cga cac atg cat ttt gat gga aca gac tac aaa gtt ctg ctc   1892
Asp Ile Arg His Met His Phe Asp Gly Thr Asp Tyr Lys Val Leu Leu
        500                 505                 510

agc cgg cag atg gga atg gtt ttt gcc ttg gat tat gac cct gtg gaa   1940
Ser Arg Gln Met Gly Met Val Phe Ala Leu Asp Tyr Asp Pro Val Glu
    515                 520                 525

agc aag ata tat ttt gca cag aca gcc ctg aag tgg ata gag agg gct   1988
Ser Lys Ile Tyr Phe Ala Gln Thr Ala Leu Lys Trp Ile Glu Arg Ala
530                 535                 540                 545

aat atg gat ggg tcc cag cga gaa aga ctg atc aca gaa gga gta gat   2036
Asn Met Asp Gly Ser Gln Arg Glu Arg Leu Ile Thr Glu Gly Val Asp
                550                 555                 560

acg ctt gaa ggt ctt gcc ctg gac tgg att ggc cgg aga atc tac tgg   2084
Thr Leu Glu Gly Leu Ala Leu Asp Trp Ile Gly Arg Arg Ile Tyr Trp
            565                 570                 575

aca gac agt ggg aag tct gtt gtt gga ggg agc gat ctg agc ggg aag   2132
Thr Asp Ser Gly Lys Ser Val Val Gly Gly Ser Asp Leu Ser Gly Lys
        580                 585                 590

cat cat cga ata atc atc cag gag aga atc tcg agg ccg cga gga ata   2180
His His Arg Ile Ile Ile Gln Glu Arg Ile Ser Arg Pro Arg Gly Ile
    595                 600                 605

gct gtg cat cca agg gcc agg aga ctg ttc tgg acg gac gta ggg atg   2228
Ala Val His Pro Arg Ala Arg Arg Leu Phe Trp Thr Asp Val Gly Met
610                 615                 620                 625

tct cca cgg att gaa agc gct tcc ctt caa ggt tcc gac cgg gtg ctg   2276
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Ser Pro Arg Ile Glu Ser Ala Ser Leu Gln Gly Ser Asp Arg Val Leu
                630                 635                 640

ata gcc agc tcc aat cta ctg gaa ccc agt gga atc acg att gac tac   2324
Ile Ala Ser Ser Asn Leu Leu Glu Pro Ser Gly Ile Thr Ile Asp Tyr
            645                 650                 655

tta aca gac act ttg tac tgg tgt gac acc aag agg tct gtg att gaa   2372
Leu Thr Asp Thr Leu Tyr Trp Cys Asp Thr Lys Arg Ser Val Ile Glu
        660                 665                 670

atg gcc aat ctg gat ggc tcc aaa cgc cga aga ctt atc cag aac gac   2420
Met Ala Asn Leu Asp Gly Ser Lys Arg Arg Arg Leu Ile Gln Asn Asp
    675                 680                 685

gta ggt cac ccc ttc tct cta gcc gtg ttt gag gat cac ctg tgg gtc   2468
Val Gly His Pro Phe Ser Leu Ala Val Phe Glu Asp His Leu Trp Val
690                 695                 700                 705

tcg gat tgg gct atc cca tcg gta ata agg gtg aac aag agg act ggc   2516
Ser Asp Trp Ala Ile Pro Ser Val Ile Arg Val Asn Lys Arg Thr Gly
                710                 715                 720

caa aac agg gta cgt ctt caa ggc agc atg ctg aag ccc tcg tca ctg   2564
Gln Asn Arg Val Arg Leu Gln Gly Ser Met Leu Lys Pro Ser Ser Leu
            725                 730                 735

gtt gtg gtc cat cca ttg gca aaa cca ggt gca gat ccc tgc tta tac   2612
Val Val Val His Pro Leu Ala Lys Pro Gly Ala Asp Pro Cys Leu Tyr
        740                 745                 750

agg aat gga ggc tgt gaa cac atc tgc caa gag agc ctg ggc aca gct   2660
Arg Asn Gly Gly Cys Glu His Ile Cys Gln Glu Ser Leu Gly Thr Ala
    755                 760                 765

cgg tgt ttg tgt cgt gaa ggt ttt gtg aag gcc tgg gat ggg aaa atg   2708
Arg Cys Leu Cys Arg Glu Gly Phe Val Lys Ala Trp Asp Gly Lys Met
770                 775                 780                 785

tgt ctc cct cag gat tat cca atc ctg tca ggt gaa aat gct gat ctt   2756
Cys Leu Pro Gln Asp Tyr Pro Ile Leu Ser Gly Glu Asn Ala Asp Leu
                790                 795                 800

agt aaa gag gtg aca tca ctg agc aac tcc act cag gct gaa gta cca   2804
Ser Lys Glu Val Thr Ser Leu Ser Asn Ser Thr Gln Ala Glu Val Pro
            805                 810                 815

gac gat gat ggg aca gaa tct tcc aca cta gtg gct gaa atc atg gtg   2852
Asp Asp Asp Gly Thr Glu Ser Ser Thr Leu Val Ala Glu Ile Met Val
        820                 825                 830

tca ggc atg aac tat gaa gat gac tgt ggt ccc ggg ggg tgt gga agc   2900
Ser Gly Met Asn Tyr Glu Asp Asp Cys Gly Pro Gly Gly Cys Gly Ser
    835                 840                 845

cat gct cga tgc gtt tca gac gga gag act gct gag tgt cag tgt ctg   2948
His Ala Arg Cys Val Ser Asp Gly Glu Thr Ala Glu Cys Gln Cys Leu
850                 855                 860                 865

aaa ggg ttt gcc agg gat gga aac ctg tgt tct gat ata gat gag tgt   2996
Lys Gly Phe Ala Arg Asp Gly Asn Leu Cys Ser Asp Ile Asp Glu Cys
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                870                 875                 880

gtg ctg gct aga tcg gac tgc ccc agc acc tcg tcc agg tgc atc aac   3044
Val Leu Ala Arg Ser Asp Cys Pro Ser Thr Ser Ser Arg Cys Ile Asn
            885                 890                 895

act gaa ggt ggc tac gtc tgc aga tgc tca gaa ggc tac gaa gga gac   3092
Thr Glu Gly Gly Tyr Val Cys Arg Cys Ser Glu Gly Tyr Glu Gly Asp
        900                 905                 910

ggg atc tcc tgt ttc gat att gac gag tgc cag cgg ggg gcg cac aac   3140
Gly Ile Ser Cys Phe Asp Ile Asp Glu Cys Gln Arg Gly Ala His Asn
    915                 920                 925

tgc gct gag aat gcc gcc tgc acc aac acg gag gga ggc tac aac tgc   3188
Cys Ala Glu Asn Ala Ala Cys Thr Asn Thr Glu Gly Gly Tyr Asn Cys
930                 935                 940                 945

acc tgc gca ggc cgc cca tcc tcg ccc gga cgg agt tgc cct gac tct   3236
Thr Cys Ala Gly Arg Pro Ser Ser Pro Gly Arg Ser Cys Pro Asp Ser
                950                 955                 960

acc gca ccc tct ctc ctt ggg gaa gat ggc cac cat ttg gac cga aat   3284
Thr Ala Pro Ser Leu Leu Gly Glu Asp Gly His His Leu Asp Arg Asn
            965                 970                 975

agt tat cca gga tgc cca tcc tca tat gat gga tac tgc ctc aat ggt   3332
Ser Tyr Pro Gly Cys Pro Ser Ser Tyr Asp Gly Tyr Cys Leu Asn Gly
        980                 985                 990

ggc gtg tgc atg cat att gaa tca ctg gac agc tac aca tgc aac tgt   3380
Gly Val Cys Met His Ile Glu Ser Leu Asp Ser Tyr Thr Cys Asn Cys
    995                1000                1005

gtt att ggc tat tct ggg gat cga tgt cag act cga gac cta cga tgg   3428
Val Ile Gly Tyr Ser Gly Asp Arg Cys Gln Thr Arg Asp Leu Arg Trp
1010                1015                1020                1025

tgg gag ctg cgt cat gct ggc tac ggg cag aag cat gac atc atg gtg   3476
Trp Glu Leu Arg His Ala Gly Tyr Gly Gln Lys His Asp Ile Met Val
                1030                1035                1040

gtg gct gtc tgc atg gtg gca ctg gtc ctg ctg ctc ctc ttg ggg atg   3524
Val Ala Val Cys Met Val Ala Leu Val Leu Leu Leu Leu Leu Gly Met
            1045                1050                1055

tgg ggg act tac tac tac agg act cgg aag cag cta tca aac ccc cca   3572
Trp Gly Thr Tyr Tyr Tyr Arg Thr Arg Lys Gln Leu Ser Asn Pro Pro
        1060                1065                1070

aag aac cct tgt gat gag cca agc gga agt gtg agc agc agc ggg ccc   3620
Lys Asn Pro Cys Asp Glu Pro Ser Gly Ser Val Ser Ser Ser Gly Pro
    1075                1080                1085

gac agc agc agc ggg gca gct gtg gct tct tgt ccc caa cct tgg ttt   3668
Asp Ser Ser Ser Gly Ala Ala Val Ala Ser Cys Pro Gln Pro Trp Phe
1090                1095                1100                1105

gtg gtc cta gag aaa cac caa gac ccc aag aat ggg agt ctg cct gcg   3716
Val Val Leu Glu Lys His Gln Asp Pro Lys Asn Gly Ser Leu Pro Ala
                1110                1115                1120
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gat ggt acg aat ggt gca gta gta gat gct ggc ctg tct ccc tcc ctg   3764
Asp Gly Thr Asn Gly Ala Val Val Asp Ala Gly Leu Ser Pro Ser Leu
            1125                1130                1135

cag ctc ggg tca gtg cat ctg act tca tgg aga cag aag ccc cac ata   3812
Gln Leu Gly Ser Val His Leu Thr Ser Trp Arg Gln Lys Pro His Ile
        1140                1145                1150

gat gga atg ggc aca ggg caa agc tgc tgg att cca cca tca agt gac   3860
Asp Gly Met Gly Thr Gly Gln Ser Cys Trp Ile Pro Pro Ser Ser Asp
    1155                1160                1165

aga gga ccc cag gaa ata gag gga aac tcc cac cta ccc tcc tac aga   3908
Arg Gly Pro Gln Glu Ile Glu Gly Asn Ser His Leu Pro Ser Tyr Arg
1170                1175                1180                1185

cct gtg ggg ccg gag aag ctg cat tct ctc cag tca gct aat gga tcg   3956
Pro Val Gly Pro Glu Lys Leu His Ser Leu Gln Ser Ala Asn Gly Ser
                1190                1195                1200

tgt cac gaa agg gct cca gac ctg cca cgg cag aca gag cca gtt aag   4004
Cys His Glu Arg Ala Pro Asp Leu Pro Arg Gln Thr Glu Pro Val Lys
            1205                1210                1215

tag aaactgggag tagacagaag gtacagaagg gaaaataaca aaccaggctg        4057


atgatggtag agtgctacag acttggtact ccagtttcca cggctaatca ctgctcgctc 4117

agggtcctga agatagctgc acagctgcag agctgcacag cgggatagct gcgacttttg 4177

cttcttgctt taagcagttc cactgaagat actcaaaaga gaagtggaga aaatcattag 4237

aaaccaaagt caagacattc atatataagc tgtgtcttct tcactggacg gtttgcctct 4297

tttccttttg cctcagaagg agtgggttaa agcaggtgac cccatgctct gtcaacccct 4357

gaataaatga tgtgatctac atagaagtct tagctcactc tcaggaacgc ttggaacact 4417

ataacttttg ctatgatata ctgccaagtg tggcccatgc tcataattgt gccttctgaa 4477

ttgtgataaa ttagtgaaaa aactgtaact tagaatctga tttattcagg attagatcat 4537

ctttttatac tataaaaatc ttcgaatgaa aatatttaac tttaaaaaca ttaccttaat 4597

cattgtcttt tcttcttgaa gtctttccca gtgaaaacgc tcaattctgc tgtttccata 4657

gaatttttaa tttattttaa gacatgagat tgtaaacaaa ttgcttgatt tattttatcc 4717

taattattta aataaaatca ccctaaagca tc                               4749
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<213> Mus musculus
<223> Murine EGF

<400> 28
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            20                  25                  30
Asn Cys Gln Pro Gly Pro Leu Glu Arg Ser Glu Arg Ser Gly Thr Cys
        35                  40                  45
Ala Gly Pro Ala Pro Phe Leu Val Phe Ser Gln Gly Lys Ser Ile Ser
    50                  55                  60
Arg Ile Asp Pro Asp Gly Thr Asn His Gln Gln Leu Val Val Asp Ala
65                  70                  75                  80
Gly Ile Ser Ala Asp Met Asp Ile His Tyr Lys Lys Glu Arg Leu Tyr
                85                  90                  95
Trp Val Asp Val Glu Arg Gln Val Leu Leu Arg Val Phe Leu Asn Gly
            100                 105                 110
Thr Gly Leu Glu Lys Val Cys Asn Val Glu Arg Lys Val Ser Gly Leu
        115                 120                 125
Ala Ile Asp Trp Ile Asp Asp Glu Val Leu Trp Val Asp Gln Gln Asn
    130                 135                 140
Gly Val Ile Thr Val Thr Asp Met Thr Gly Lys Asn Ser Arg Val Leu
145                 150                 155                 160
Leu Ser Ser Leu Lys His Pro Ser Asn Ile Ala Val Asp Pro Ile Glu
                165                 170                 175
Arg Leu Met Phe Trp Ser Ser Glu Val Thr Gly Ser Leu His Arg Ala
            180                 185                 190
His Leu Lys Gly Val Asp Val Lys Thr Leu Leu Glu Thr Gly Gly Ile
        195                 200                 205
Ser Val Leu Thr Leu Asp Val Leu Asp Lys Arg Leu Phe Trp Val Gln
    210                 215                 220
Asp Ser Gly Glu Gly Ser His Ala Tyr Ile His Ser Cys Asp Tyr Glu
225                 230                 235                 240
Gly Gly Ser Val Arg Leu Ile Arg His Gln Ala Arg His Ser Leu Ser
                245                 250                 255
Ser Met Ala Phe Phe Gly Asp Arg Ile Phe Tyr Ser Val Leu Lys Ser
            260                 265                 270
Lys Ala Ile Trp Ile Ala Asn Lys His Thr Gly Lys Asp Thr Val Arg
        275                 280                 285
Ile Asn Leu His Pro Ser Phe Val Thr Pro Gly Lys Leu Met Val Val
    290                 295                 300
His Pro Arg Ala Gln Pro Arg Thr Glu Asp Ala Ala Lys Asp Pro Asp
305                 310                 315                 320
Pro Glu Leu Leu Lys Gln Arg Gly Arg Pro Cys Arg Phe Gly Leu Cys
                325                 330                 335
Glu Arg Asp Pro Lys Ser His Ser Ser Ala Cys Ala Glu Gly Tyr Thr
            340                 345                 350
Leu Ser Arg Asp Arg Lys Tyr Cys Glu Asp Val Asn Glu Cys Ala Thr
        355                 360                 365
Gln Asn His Gly Cys Thr Leu Gly Cys Glu Asn Thr Pro Gly Ser Tyr
    370                 375                 380
His Cys Thr Cys Pro Thr Gly Phe Val Leu Leu Pro Asp Gly Lys Gln
385                 390                 395                 400
Cys His Glu Leu Val Ser Cys Pro Gly Asn Val Ser Lys Cys Ser His
                405                 410                 415
Gly Cys Val Leu Thr Ser Asp Gly Pro Arg Cys Ile Cys Pro Ala Gly
            420                 425                 430
Ser Val Leu Gly Arg Asp Gly Lys Thr Cys Thr Gly Cys Ser Ser Pro
        435                 440                 445
Asp Asn Gly Gly Cys Ser Gln Ile Cys Leu Pro Leu Arg Pro Gly Ser
    450                 455                 460
Trp Glu Cys Asp Cys Phe Pro Gly Tyr Asp Leu Gln Ser Asp Arg Lys
465                 470                 475                 480
Ser Cys Ala Ala Ser Gly Pro Gln Pro Leu Leu Leu Phe Ala Asn Ser
                485                 490                 495
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Gln Asp Ile Arg His Met His Phe Asp Gly Thr Asp Tyr Lys Val Leu
            500                 505                 510
Leu Ser Arg Gln Met Gly Met Val Phe Ala Leu Asp Tyr Asp Pro Val
        515                 520                 525
Glu Ser Lys Ile Tyr Phe Ala Gln Thr Ala Leu Lys Trp Ile Glu Arg
    530                 535                 540
Ala Asn Met Asp Gly Ser Gln Arg Glu Arg Leu Ile Thr Glu Gly Val
545                 550                 555                 560
Asp Thr Leu Glu Gly Leu Ala Leu Asp Trp Ile Gly Arg Arg Ile Tyr
                565                 570                 575
Trp Thr Asp Ser Gly Lys Ser Val Val Gly Gly Ser Asp Leu Ser Gly
            580                 585                 590
Lys His His Arg Ile Ile Ile Gln Glu Arg Ile Ser Arg Pro Arg Gly
        595                 600                 605
Ile Ala Val His Pro Arg Ala Arg Arg Leu Phe Trp Thr Asp Val Gly
    610                 615                 620
Met Ser Pro Arg Ile Glu Ser Ala Ser Leu Gln Gly Ser Asp Arg Val
625                 630                 635                 640
Leu Ile Ala Ser Ser Asn Leu Leu Glu Pro Ser Gly Ile Thr Ile Asp
                645                 650                 655
Tyr Leu Thr Asp Thr Leu Tyr Trp Cys Asp Thr Lys Arg Ser Val Ile
            660                 665                 670
Glu Met Ala Asn Leu Asp Gly Ser Lys Arg Arg Arg Leu Ile Gln Asn
        675                 680                 685
Asp Val Gly His Pro Phe Ser Leu Ala Val Phe Glu Asp His Leu Trp
    690                 695                 700
Val Ser Asp Trp Ala Ile Pro Ser Val Ile Arg Val Asn Lys Arg Thr
705                 710                 715                 720
Gly Gln Asn Arg Val Arg Leu Gln Gly Ser Met Leu Lys Pro Ser Ser
                725                 730                 735
Leu Val Val Val His Pro Leu Ala Lys Pro Gly Ala Asp Pro Cys Leu
            740                 745                 750
Tyr Arg Asn Gly Gly Cys Glu His Ile Cys Gln Glu Ser Leu Gly Thr
        755                 760                 765
Ala Arg Cys Leu Cys Arg Glu Gly Phe Val Lys Ala Trp Asp Gly Lys
    770                 775                 780
Met Cys Leu Pro Gln Asp Tyr Pro Ile Leu Ser Gly Glu Asn Ala Asp
785                 790                 795                 800
Leu Ser Lys Glu Val Thr Ser Leu Ser Asn Ser Thr Gln Ala Glu Val
                805                 810                 815
Pro Asp Asp Asp Gly Thr Glu Ser Ser Thr Leu Val Ala Glu Ile Met
            820                 825                 830
Val Ser Gly Met Asn Tyr Glu Asp Asp Cys Gly Pro Gly Gly Cys Gly
        835                 840                 845
Ser His Ala Arg Cys Val Ser Asp Gly Glu Thr Ala Glu Cys Gln Cys
    850                 855                 860
Leu Lys Gly Phe Ala Arg Asp Gly Asn Leu Cys Ser Asp Ile Asp Glu
865                 870                 875                 880
Cys Val Leu Ala Arg Ser Asp Cys Pro Ser Thr Ser Ser Arg Cys Ile
                885                 890                 895
Asn Thr Glu Gly Gly Tyr Val Cys Arg Cys Ser Glu Gly Tyr Glu Gly
            900                 905                 910
Asp Gly Ile Ser Cys Phe Asp Ile Asp Glu Cys Gln Arg Gly Ala His
        915                 920                 925
Asn Cys Ala Glu Asn Ala Ala Cys Thr Asn Thr Glu Gly Gly Tyr Asn
    930                 935                 940
Cys Thr Cys Ala Gly Arg Pro Ser Ser Pro Gly Arg Ser Cys Pro Asp
945                 950                 955                 960
Ser Thr Ala Pro Ser Leu Leu Gly Glu Asp Gly His His Leu Asp Arg
                965                 970                 975
Asn Ser Tyr Pro Gly Cys Pro Ser Ser Tyr Asp Gly Tyr Cys Leu Asn
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```
          980                 985                 990
Gly Gly Val Cys Met His Ile Glu Ser Leu Asp Ser Tyr Thr Cys Asn
        995                1000                1005
Cys Val Ile Gly Tyr Ser Gly Asp Arg Cys Gln Thr Arg Asp Leu Arg
   1010                1015                1020
Trp Trp Glu Leu Arg His Ala Gly Tyr Gly Gln Lys His Asp Ile Met
1025               1030                1035                1040
Val Val Ala Val Cys Met Val Ala Leu Val Leu Leu Leu Leu Leu Gly
                1045                1050                1055
Met Trp Gly Thr Tyr Tyr Tyr Arg Thr Arg Lys Gln Leu Ser Asn Pro
            1060                1065                1070
Pro Lys Asn Pro Cys Asp Glu Pro Ser Gly Ser Val Ser Ser Ser Gly
        1075                1080                1085
Pro Asp Ser Ser Ser Gly Ala Ala Val Ala Ser Cys Pro Gln Pro Trp
   1090                1095                1100
Phe Val Val Leu Glu Lys His Gln Asp Pro Lys Asn Gly Ser Leu Pro
1105               1110                1115                1120
Ala Asp Gly Thr Asn Gly Ala Val Val Asp Ala Gly Leu Ser Pro Ser
                1125                1130                1135
Leu Gln Leu Gly Ser Val His Leu Thr Ser Trp Arg Gln Lys Pro His
            1140                1145                1150
Ile Asp Gly Met Gly Thr Gly Gln Ser Cys Trp Ile Pro Pro Ser Ser
        1155                1160                1165
Asp Arg Gly Pro Gln Glu Ile Glu Gly Asn Ser His Leu Pro Ser Tyr
   1170                1175                1180
Arg Pro Val Gly Pro Glu Lys Leu His Ser Leu Gln Ser Ala Asn Gly
1185               1190                1195                1200
Ser Cys His Glu Arg Ala Pro Asp Leu Pro Arg Gln Thr Glu Pro Val
                1205                1210                1215
Lys
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<210> 29
<211> 4119
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (32)..(514)

<220>
<223> Human TGF- alpha

<400> 29

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                                     Met Val Pro Ser Ala Gly Gln
                                       1               5

ctc gcc ctg ttc gct ctg ggt att gtg ttg gct gcg tgc cag gcc ttg   100
Leu Ala Leu Phe Ala Leu Gly Ile Val Leu Ala Ala Cys Gln Ala Leu
         10                  15                  20

gag aac agc acg tcc ccg ctg agt gca gac ccg ccc gtg gct gca gca   148
Glu Asn Ser Thr Ser Pro Leu Ser Ala Asp Pro Pro Val Ala Ala Ala
     25                  30                  35

gtg gtg tcc cat ttt aat gac tgc cca gat tcc cac act cag ttc tgc   196
Val Val Ser His Phe Asn Asp Cys Pro Asp Ser His Thr Gln Phe Cys
 40                  45                  50                  55
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ttc cat gga acc tgc agg ttt ttg gtg cag gag gac aag cca gca tgt   244
Phe His Gly Thr Cys Arg Phe Leu Val Gln Glu Asp Lys Pro Ala Cys
                60                  65                  70

gtc tgc cat tct ggg tac gtt ggt gca cgc tgt gag cat gcg gac ctc   292

Val Cys His Ser Gly Tyr Val Gly Ala Arg Cys Glu His Ala Asp Leu
            75                  80                  85

ctg gcc gtg gtg gct gcc agc cag aag aag cag gcc atc acc gcc ttg   340
Leu Ala Val Val Ala Ala Ser Gln Lys Lys Gln Ala Ile Thr Ala Leu
        90                  95                  100

gtg gtg gtc tcc atc gtg gcc ctg gct gtc ctt atc atc aca tgt gtg   388
Val Val Val Ser Ile Val Ala Leu Ala Val Leu Ile Ile Thr Cys Val
    105                 110                 115

ctg ata cac tgc tgc cag gtc cga aaa cac tgt gag tgg tgc cgg gcc   436
Leu Ile His Cys Cys Gln Val Arg Lys His Cys Glu Trp Cys Arg Ala
120                 125                 130                 135

ctc atc tgc cgg cac gag aag ccc agc gcc ctc ctg aag gga aga acc   484
Leu Ile Cys Arg His Glu Lys Pro Ser Ala Leu Leu Lys Gly Arg Thr
            140                 145                 150

gct tgc tgc cac tca gaa aca gtg gtc tga agagcccaga ggaggagttt     534
Ala Cys Cys His Ser Glu Thr Val Val
        155                 160

ggccaggtgg actgtggcag atcaataaag aaaggcttct tcaggacagc actgccagag 594

atgcctgggt gtgccacaga ccttcctact tggcctgtaa tcacctgtgc agccttttgt 654

gggccttcaa aactctgtca agaactccgt ctgcttgggg ttattcagtg tgacctagag 714

aagaaatcag cggaccacga tttcaagact tgttaaaaaa gaactgcaaa gagacggact 774

cctgttcacc taggtgaggt gtgtgcagca gttggtgtct gagtccacat gtgtgcagtt 834

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tggggaagaa agtgaaggag gggaaactgt ttaatatcac atgaagaccc tagctttaag 1014

agaagctgta tcctctaacc acgagactct caaccagccc aacatcttcc atggacacat 1074

gacattgaag accatcccaa gctatcgcca cccttggaga tgatgtctta tttattagat 1134

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agccggtaaa tgcctcaata cgttctggga gaaaacttag caaatccatc agcagggatc 1434
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tgtcccctct gttggggaga gaggaagagt gtgtgtgtct acacaggata aacccaatac 1494

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ttagaaatag aacattagcc acgagccata ggcatttcag gccaaatcca tgaaaggggg 1614

accagtcatt tattttccat tttgttgctt ggttggtttg ttgctttatt tttaaaagga 1674

gaagtttaac tttgctattt attttcgagc actaggaaaa ctattccagt aatttttttt 1734

tcctcatttc cattcaggat gccggcttta ttaacaaaaa ctctaacaag tcacctccac 1794

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gctgaccccc tagcaacgta gttgacacag ctccaggttt ttaaattact aaaataagtt 2334

caagtttaca tcccttgggc cagatatgtg ggttgaggct tgactgtagc atcctgctta 2394

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cagtgccctc atccttatgc ctgtaacaca catgcagtga gaggcctcac atatacgcct 2634

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taaaatggtc aacttttcat tatctttatt ataataaacc tgatgctttt ttttagaact 3834

ccttactctg atgtctgtat atgttgcact gaaaaggtta atatttaatg ttttaattta 3894

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cctttttttc ctaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 4119

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<211> 160
<212> PRT
<213> Homo sapiens
<223> Human TGF- alpha

<400> 30

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             20                  25                  30
Asp Pro Pro Val Ala Ala Ala Val Val Ser His Phe Asn Asp Cys Pro
         35                  40                  45
Asp Ser His Thr Gln Phe Cys Phe His Gly Thr Cys Arg Phe Leu Val
     50                  55                  60
Gln Glu Asp Lys Pro Ala Cys Val Cys His Ser Gly Tyr Val Gly Ala
 65                  70                  75                  80
Arg Cys Glu His Ala Asp Leu Leu Ala Val Val Ala Ala Ser Gln Lys
                 85                  90                  95
Lys Gln Ala Ile Thr Ala Leu Val Val Val Ser Ile Val Ala Leu Ala
            100                 105                 110
Val Leu Ile Ile Thr Cys Val Leu Ile His Cys Cys Gln Val Arg Lys
        115                 120                 125
His Cys Glu Trp Cys Arg Ala Leu Ile Cys Arg His Glu Lys Pro Ser
    130                 135                 140
Ala Leu Leu Lys Gly Arg Thr Ala Cys Cys His Ser Glu Thr Val Val
145                 150                 155                 160
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<210> 31

<211> 3776
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (91)..(570)

<220>
<223> Murine TGF-alpha

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Met Val Pro Ala Thr Gly Gln Leu
1 5

gct ctg cta gcg ctg ggt atc ctg tta gct gtg tgc cag gct ctg gag 162
Ala Leu Leu Ala Leu Gly Ile Leu Leu Ala Val Cys Gln Ala Leu Glu
10 15 20

aac agc aca tcc ccc ctg agt gac tca ccc gtg gcg gct gca gtg gtg 210
Asn Ser Thr Ser Pro Leu Ser Asp Ser Pro Val Ala Ala Ala Val Val
25 30 35 40

tct cac ttc aac aag tgc cca gat tcc cac act cag tac tgc ttc cat 258
Ser His Phe Asn Lys Cys Pro Asp Ser His Thr Gln Tyr Cys Phe His
45 50 55

gga acc tgc cgg ttt ttg gtg cag gaa gag aag cca gca tgt gtc tgc 306
Gly Thr Cys Arg Phe Leu Val Gln Glu Glu Lys Pro Ala Cys Val Cys
60 65 70

cac tct ggg tac gtg ggt gtt cgc tgt gag cat gca gac ctc ctg gct 354
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2003/000949

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 14/71, A61K 38/17, A61P 25/28, A61P 25/02, A61P 35/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOSIS, Accession number PREV200300324932, Kuja-Panula J et al, "Amigo1-A transmembrane protein implicated in fiber tract development defines a novel protein family with leucine- rich repeats", Society for Neuroscience Abstract Viewer and Itinerary Planner, 2002, Vol. 2002, Abstract No. 628.17	13-14,16-36, 38-47,57-58
A	--	1-12,15,37, 48-56,59-61

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 February 2004	19 -02- 2004
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer IDA CHRISTENSEN/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2003/000949

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Molecular Biology of the Cell, Volume 12, Nr. Supplement, November 2001, "Cloning and biological properties of a novel LRRs (leucine-rich repeats) containing protein induced on amphoterin matrix (AMIGO)", pages 192a-193a	13-14,16-36, 38-47,57-58
A	--	1-12,15,37, 48-56,59-61
E,X	WO 2004003165 A2 (IMCLONE SYSTEMS INCORPORATED), 8 January 2004 (08.01.2004), The whole document & STN International, File Registry, RN 640808-22-6 (human clone S30-21616/DEGA), 100% identity in 522 aa overlap with SEQ.ID.No. 4	1-36,38-58
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WO	2004003165	A2	08/01/2004	NONE

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2003/000949

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☐ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☐ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2003/000949

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 21, 38-45, 47-57, 59-61 (entirely), 25 (partially)
because they relate to subject matter not required to be searched by this Authority, namely:
see extra sheet

2. ☒ Claims Nos.: 13-14, 16-17, 20, 38, 48 (partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see extra sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Box II.1

Claims: 21, 38-45, 47-57, 59-61 (entirely), 25 (partially)

Claims 21, 38-45, 47-57 and 59-61 relate to methods of treatment of the human or animal body by therapy. Claim 25 may relate to a method of treatment of the human or animal body by therapy (PCT Rule 39.1(iv)). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds or compositions.

Box II.2

Claims: 13-14, 16-17, 20, 38, 48 (partially)

Present claims 13-14 and 16-17 relate to a kit comprising a compound defined by reference to a desirable characteristic or property, namely being capable of detecting AMIGO or allelic variants thereof. The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and / or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. Additionally, previously known compounds may be included in the scope of the present claims. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.
Consequently, the search of claims 13-14 and 16-17 has been limited to the general aspects of kits comprising primers or probes, which may detect AMIGO.

.../...

Claim 20 relates in part to a compound defined by reference to a desirable characteristic or property, namely being an AMIGO agonist/antagonist. The claim covers all compounds having this characteristic or property, whereas the application lacks support for such compounds. Additionally, previously known compounds may be included in the scope of the present claim. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search of claim 20 has not covered AMIGO agonists or antagonists.

Claims 38 and 48 in part relate to a molecule defined by reference to a desirable characteristic or property, namely that selectively inhibits AMIGO binding to the AMIGO receptor (with or without binding to the EGFR receptor). The claims cover all compounds having this characteristic or property, whereas the application lacks support for such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search of claims 38 and 48 has not covered steps (b)(v) and (b)(vi).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2003/000949

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

Transmembrane proteins AMIGO, AMIGO2 and AMIGO3 (Amphoterin induced gene and orphan receptor), wherein the extracellular part contains six leucine-rich repeats (LRRs) and one immunoglobulin domain. Use of said proteins for modulating growth, migration, axonal growth, myelination, fasciculation or proliferation of neuronal cells and for treating cancer, tumour growth or metastasis. Methods of screening for agents which modulate the interaction between two AMIGO compounds or between AMIGO and epidermal growth factor receptor (EGFR).